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4. Prof. Girjesh Govil, Ph.D., F.N.A., F.A.Sc., F.T.W.A.S., Mumbai.

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EDITORS' PAGE

There is a News Item in the June 2004 issue of Materials Today (page 23) inserted by its Editor, Cordelia Sealy, regarding funding in research in France. This seems quite relevant in the context of India's research in general and teaching posts in Universities/Colleges which have recently started operating on low paid fixed term contract teachers. Therefore, that particular News Item is quoted here; **France Saves Research.** *Researchers are celebrating as the French Government announces measures that meet the demands of 'Sauvons la Recherche' (Let's Save Research). The campaign began in January to protest at the lack of funds for basic research and the downgrading of many public research posts to fixed-term contracts. Over 70,000 researchers signed an open letter to the government, and, despite an initial the offer, over 3500 research unit director and specialist team leaders resigned from their administrative roles on March 9th. Francois Fillon, the new French Education Minister, announced the creation of 550 new research jobs in public institutions and 1050 teaching posts in Universities. 'The government has decided on an exceptional and immediate effort in favour of scientific jobs in the benefit of public research', he stated. The additional University teaching posts were essential to the favourable solution, said Fillon.*"

This News Item tells us that there are at least some progressive looking governments in the world who value University Research. However, the expediency may have been the result of a compaign from laboratory and university scientists. Can our scientists and scientific administrators/Directors show the same kinship as shown by their French counterparts. Possibly not!

Girjesh Govil
Jai Pal Mittal
Suresh Chandra

The views expressed here are solely those of one of the Editors and do not necessarily reflect those of the Academy or the Institute where he works.

Metabonomics: A new frontier of nuclear magnetic resonance (NMR)*

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Abstract

The functional information of living systems is coded in their genome. However, its expression in the form of metabolism is controlled by a number of factors. Metabolism is therefore the final frontier towards understanding the function of a cell, organ or an individual. Such studies are crucial in understanding human behavior, health and diseases. Metabolic studies at the level of cells, tissues and whole organs have become an important field of biological and biomedical research. A new term metabonomics has been coined for such studies.

Fourier Transform (FT) NMR has made a major impact in metabonomics. This powerful and emerging technology characterizes the complex time-dependent metabolic profiles in bio-fluids, cells, tissues and organs and helps in following dynamic chemical changes in organisms. It has been used to provide information on drug toxicity and efficacy, clinical diagnostics, reproductive biology, and gene function. Among the NMR active nuclei useful in biological research, ^{1}H has the highest sensitivity and detects simultaneously a large number of metabolites, which may be present in living systems. ^{31}P spectra show resonances of energy rich phosphates and are used as measures of the energy-state, intra- and inter-cellular pH, as well as in identifying precursors and catabolic products of membrane lipids. In combination with isotope labeling, ^{13}C NMR plays an important role in identification and quantification of metabolic products. Use of enriched substrates allow measurement of ^{13}C spectra within short time and with good spectral resolution and has the advantage of elucidating location of the label within metabolites, and verifying unusual metabolic pathways. Isotopic data may be obtained from excreted products, cytoplasmic fluids, and from macromolecular cell constituents after prolonged incubation with the ^{13}C-labelled substrates. Several key studies have demonstrated the potential of ^{13}C labeling and NMR spectroscopy for determination of metabolic fluxes in the pathways of primary metabolism. Using sophisticated computation methods, complete metabolic networks can be analyzed from NMR data sets. High resolution, magic angle spinning and *in-vivo* localized ^{1}H NMR spectroscopy provide methods for characterizing and quantifying a wide range of metabolites in biological tissues, intact cells, and biofluids involving hundreds of endogenous metabolites, as a function of time.

Using our own work and that of others, I have illustrated, how NMR has been used to detect: *in vivo* presence of unusual or unexpected molecules;

*Prof. N.R. Dhar Memorial Award Lecture, delivered at Allahabad on July 29, 2004

changes in levels of compounds as a result of disease, action of a drug, or during storage of cells; detection of metabolites during cell activity and monitoring unusual metabolism; excretion of molecules such as drug or their metabolites in body fluids; detection of diseases, and several other applications.

(Keywords : metabonomics/ NMR/ dynamic chemical change)

Introduction

It is a great honor that the Academy has selected me to deliver Prof. N. R. Dhar memorial lecture for 2004. Prof. Dhar was one of the most outstanding chemists that our country has produced. His work on soil chemistry and allied areas received international recognition. He had written two books on Biochemistry at a time when this subject was not considered as a separate discipline in academic institutions. He was one of the Founder members of our Academy and donated the land on which the new building of the Academy stands. I also feel privileged to note that the first award in this series was given to Prof. R. C. Mehrotra, who was my teacher and mentor. We unfortunately lost him a few days back. In him, India has lost another outstanding chemist and educationalist. I would like to dedicate this lecture to the memory of Prof. Mehrotra.

Flow of information in evolved living systems such as mammals occurs from the one dimensional (1D) information present in the form of base-sequences of nucleic acids (genomics) to the 3D structures of proteins, which in turn, govern almost all activities of cells and whole organs. Though all cells of an individual are derived from the same initial genomic material (paternal gene from spermatozoa and the maternal genome from egg), cell differentiation during development leads to different expression of genes in different organs. Further, cells from the same organ may show different chemistry if subjected to stresses such as disease, chemical and environmental changes and other factors. The knowledge of DNA sequences of a large number of species including human is emerging at a relatively fast rate. However, this is only the first step to understand behavior of living systems. Knowledge of the 3D structures of proteins (proteomics), t-RNA, ribosomes and other macromolecular assemblies and understanding structure-function relationship is the next. Metabonomics is a term, which has been used to understand the behavior of cellular systems and aberrations in their functions as a result of stresses or during cell development and differentiation. It has been defined as "the quantitative measurement of the dynamic multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification". Besides understanding the basic aspects of life sciences, the most fruitful outcome of these endeavors may result in better understanding of human health and for exploring possibilities of providing better life to mankind.

NMR has been used to look at all aspects of life sciences

Nuclear Magnetic Resonance (NMR) spectroscopy has developed into a powerful technique in life sciences. Of the five major elements (C, H, N, O and P) in living systems, four have nuclei, which can be conveniently studied by NMR. However, when applying NMR to biological systems, one faces several difficulties. Foremost is the low sensitivity in NMR detection (with the present day technology, one needs mM

concentrations to observe a good ^{1}H spectrum). This is coupled with the low natural abundance of nuclei of choice for C and N (^{13}C and ^{15}N). In fact, the advances in NMR in living systems can be closely related to improvement in sensitivity in NMR detection as a result of improved electronics and probe design. One observes a large number of spectral lines from thousands of molecules in living systems leading to difficulties in resolution and assignment. While observing ^{1}H NMR, one has a huge water line (corresponding to approximately 80 M proton density), against which mM concentrations of the molecules of interest have to be observed. Finally, spectral lines in large organized systems are broad. Several of these problems have been solved through the development of Fourier Transform (FT) techniques for which the Nobel Prize for the year 1991 was awarded to Richard Ernst.

NMR applications in medicine, biochemistry and biophysics cover three diverse areas. One is study of structure and dynamics of biological macromolecules, such as proteins, carbohydrates and nucleic acids, and molecular assemblies such as biological membranes and protein-nucleic acid complexes. A few mg of pure compounds dissolved in about 0.5 ml of water is sufficient for such studies. It is advantageous to use highest achievable magnetic fields (spectrometers operating up to 21 T are commercially available), so as to obtain high sensitivity and good dispersions of the chemically shifted ^{1}H, ^{13}C, ^{15}N or ^{31}P resonances. Parameters such as chemical shifts, coupling constants, relaxation rates, solvent exchange and nuclear Overhausser effects are used to obtain structural information. Exploring 3D structures and dynamics of bio-molecules and their interaction with other molecules, is an active area of research. NMR provides information on bio-molecular structures and their interactions in aqueous solutions, where the molecules adopt somewhat more flexible structures compared to solid-state structures obtained by X-ray crystallography. Kinetic and thermodynamic parameters can be obtained when more than one chemical species coexist in solution thus providing a greater insight on the energy landscape, such as in protein unfolding. Such investigations add to the knowledge from genomics and proteomics by providing information on the structure-activity relationship of biological molecules. Kurt Wuthrich was awarded Nobel Prize for this work in 2002. It is a matter of great honor for us that both Ernst and Wuthrich are Fellows of our Academy.

A branch of NMR commonly known as magnetic resonance imaging (MRI) and spectroscopy (MRS) (the word nuclear is dropped in hospital environment for obvious reasons), has important applications in health care and in pharmaceutical and clinical research. MRI and MRS allow non-invasive measurement in living systems by providing excellent anatomical as well as metabolic information. Studies require large magnets such that whole animals and human being can be accommodated. However, relatively lower magnetic fields (1-2 T) are used to avoid any physiological or clinical damage to the body. One looks selectively at an organ (e.g. brain or heart) and maps NMR properties of a single molecule (say ^{1}H in H_2O or ^{31}P in ATP) in the form of its image in three dimensions. While X-ray CT scan is better for obtaining information on bones, NMR provides information on soft tissues. Last year's Nobel prize was shared

by Peter Mansfield and Paul Lauterbur for their pioneering work leading to development of MRI.

NMR Based Metabonomics

A relatively less explored frontier of NMR involves studies on the chemistry of living cells, and analysis of body fluids (blood plasma, urine, stool, seminal fluids etc) and tissues under different physiological and patho-physiological conditions. Cellular systems and tissues offer substantial advantages in terms of sensitivity and resolution for metabolic studies, over studies on whole organs. Unlike in the other two cases, dedicated instruments are not available for cellular studies. One generally uses high field spectrometers meant for molecular studies and design strategies to keep the cells viable while they are in the spectrometer and to prevent them from settling in the sample tube. Most cells require a well-regulated medium that include buffers, nutrients and in many cases continuous supply of oxygen. Gel thread methods are the simplest and closely resemble biological situation. It is helpful to compare the spectra of intact cells with cell free extracts, which give much sharper lines and thus, provide easier assignments.

It is possible to analyze the chemical constituents of the living cells and their metabolism using NMR. While chemical methods are available for such studies, and have been used extensively to understand biochemistry of cells, these often depend on destroying the cells during analysis and strategies are developed to look for a particular molecule. NMR studies can be conducted while the cells are alive and metabolically active, and simultaneously provide information on all metabolites. Nevertheless, it is always an advantage to supplement NMR with other physical techniques such as HPLC, MS and GCMS. The wealth of NMR information can be profitably coupled to multi-variant statistical analysis and thus correlated with stresses and diseases. Often, a great deal of information can be obtained from such empirical analysis even before the difficult process of assignment is carried out.

Cell metabolism is a highly intricate network of coupled reactions. The key pathways are glycolysis, citric acid cycle and oxidative phosphorylation. Study of cell metabolism in aerobic conditions (presence of oxygen) requires special modifications in probes and in sample handling. Inactive cells, tissues, cell free extracts, body fluids and glycolysis in cells which require anaerobic conditions, can however be studied simply by putting the sample in an NMR tube and recording high resolution NMR spectra. Using such techniques, changes in the kinetics of the metabolic processes under the influence of activators or drugs can be followed. NMR can answer some of the vital questions on the chemistry of living cells, which cannot be obtained by conventional biochemical methods. It has been used as a pathological tool to differentiate between cancerous and benign cells (biopsy) and to detect excretion of compounds by analysis of urine, blood and stool (pathology).

NMR signals from large molecular weight compounds such as proteins, nucleic acids, lipid assemblies and carbohydrates are broad and do not provide any useful information in cellular studies. However, low molecular weight compounds can be detected and their concentrations in cellular systems, body fluids and tissues can be

estimated. Typically 50-100 molecules present in mM concentrations in cellular suspensions or in body fluids can be detected. Water suppressed ^{1}H NMR can be particularly useful for detecting small molecules. A number of different approaches have been used for suppression of water signal, including WEFT, CPMG and jump and return. In view of the large number of compounds in cells, it is useful to use 2D techniques such as DQF-COSY and HSQC (^{1}H-^{1}H and ^{1}H-^{13}C correlated spectroscopy). This helps both in resolution and assignment. Analysis is aided by a library of the finger print patterns of expected compounds and confirmed by spiking techniques, where an expected compound may be added to look at change in intensities of existing peaks in the system.

NMR studies of spermatozoa

I shall illustrate the wealth of information, which can be derived from NMR using studies on spermatozoa as an example. From the view point of NMR, spermatozoa are extremely friendly as they can survive for a long time under anaerobic conditions and do not settle because of their motility. General features of sperm morphology, and its diversity in different animals has been the subject of extensive investigations using microscopic techniques. Its overall structure can be divided into three parts; (a) head, which contains the paternal chromatin and has a cap-like cover called acrosome, which participate in egg penetration and fusion (b) mid-piece containing mitochondria and which contributes to most of the NMR signals and (c) tail which is responsible for the movement in the seminal fluid (motility). Motility and fertilizing ability are the major markers of spermatozoan activity, which can be beneficially or detrimentally influenced by a variety of physical and chemical factors. Implication of such factors in fertility regulation, artificial insemination, cellular and molecular biology of spermatozoa need not be emphasized to this audience.

Epididymis, is the male reproductive organ. It acts as a highly efficient storage system and enables spermatozoa to mature and preserves/enhances their motility and fertilizing capacity. There are three main parts of epididymis: caput is the birth place of spermatozoa, corpus where they are in semi-matured stage and cauda where they are fully matured. During the passage through epididymis, spermatozoa undergo major morphological and biochemical changes. The next stages in the life cycle of spermatozoa are capacitation, a process where spermatozoa are activated and the acrosomal reaction where the cap region delivers hydrolytic enzymes so that it can fuse (fertilize) with the egg. These two processes take place in the female reproductive tract. Changes in the biochemistry of spermatozoa in all the five steps mentioned above, have been followed using NMR.

Identification of small molecular weight compounds

^{1}H and ^{13}C NMR allows direct detection of the molecules present in the cells. Cells can be fed on ^{13}C labeled substrates and metabolic pathways can be followed. For example, when cells are fed with glucose (Glc) labeled at position 1, the label appears at the corresponding carbon atoms in the various metabolites. In the normal glycolysis for example, a decrease in the glucose signal, and build up of signals from lactate (arising from C-3 methyl) can be monitored with time (Fig. 1).

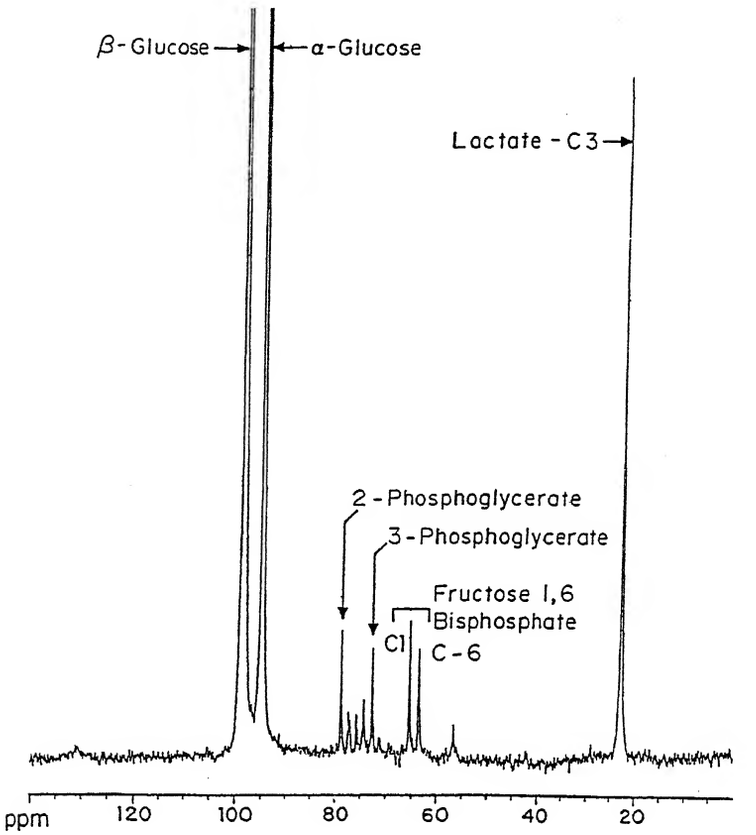

Fig. 1– Various stages of metabolism in cells can be followed using ^{13}C labeled substrates followed by ^{13}C NMR. In the figure, glycolysis by spermatozoa is monitored using glucose (Glc) labeled at position 1. Signals of both inter-converting anomeric forms of glucose are seen. The Glc signals decay with time and that of the end product lactate (Lac) increases. Signals from several of the intermediates are also visible. The kinetics of the various stages in glycolysis can be monitored and the effects of external molecules (activators and inhibitors) can be obtained.

We have typically used 10^5 cells suspended in Dulbecco medium for obtaining 2D DQF-COSY and HSQC spectra (Fig. 2). The wealth of information available in such spectra is obvious. The composition and concentration of metabolites changes dramatically, not only from species to species, but also within samples from the same group of animals. The signals and levels of several amino acids such as Ala, Arg, Asp, Gly, Glu, Ile, Leu, Lys, Met, Pro, Thr, Val, molecules such as Lactate (Lac), m-inositol, glycerophosphorylcholine (GPC), hypotaurine and aromatic molecules such as uridinediphosphoglucose (UDP) could thus be identified and their concentrations measured. The relative concentrations of these molecules change as a result of maturation. Two rather unexpected molecules detected by NMR were β-Ala and hypotaurine, which have been reported for the first time in goat spermatozoa (Fig. 2).

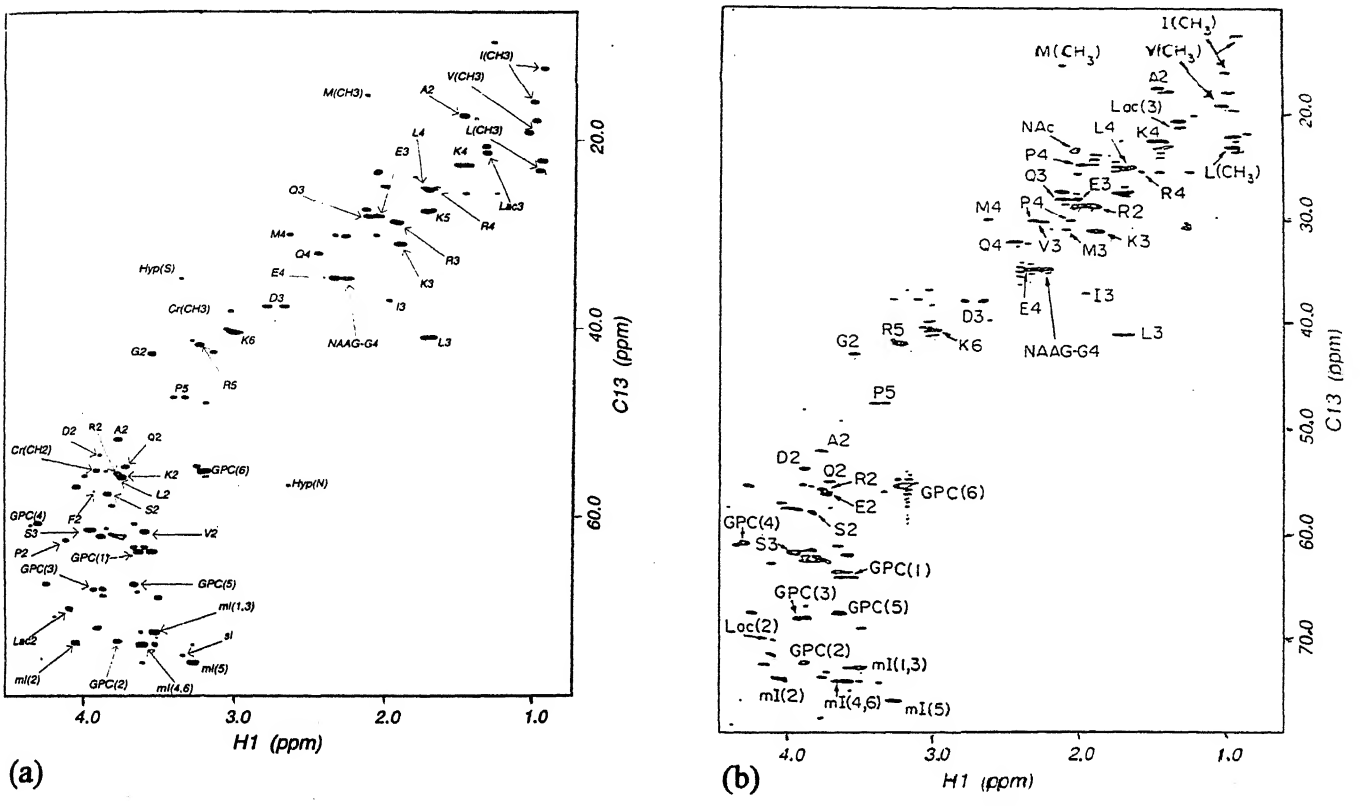

Fig. 2– Two-dimensional (2D) NMR techniques help in not only better dispersion but also in assignments of various molecules in complex systems. In spermatozoa (as in other body fluids) almost fifty different molecules can be identified and their levels detected. Figure shows the upfield region of the gradient-enhanced ^{1}H-^{13}C (J-corrleated) HSQC spectrum of spermatozoa from two different regions of goat epididymis. (a) cadua region and (b) caput region. The spectra are using natural abundance of ^{13}C. Note that hypotaurine required for cell maturation is present in less matured cells (caput region) but its synthesis is switched off once the cells mature. The levels of several other molecules also change with cell maturation.

Metabolism in cells

Cells derive their energy from ATP, which in turn is synthesized by biochemical pathways mentioned, earlier. The overall reactions in glycolyis under anaerobic conditions can be represented by:

$$Glc + 2ADP + 2P_i =$$

$$2Lac + 2ATP + 2H^+ + 2H_2O$$

Thus, the overall metabolism can be followed by decrease in the ^{13}C (Glc) signal or an increase of ^{13}C (Lac) signal. ^{31}P NMR is particularly useful to detect ADP and ATP levels and intra and inter cellular pH (Fig. 3). There are changes in ^{31}P signals for ATP, ADP and P_i and other phosphorus containing molecules. Using these parameters, effect of various external factors on glycolysis can be determined. In the case of spermatozoa, the energy released in glycolysis and trapped in the form of ATP is needed for its biochemical requirements, maturation, capacitation, motility and fertilization. Cells, which are dormant, (for example, when stored under frozen conditions or starved) have a low level of ATP and higher level of ADP and P_i. When

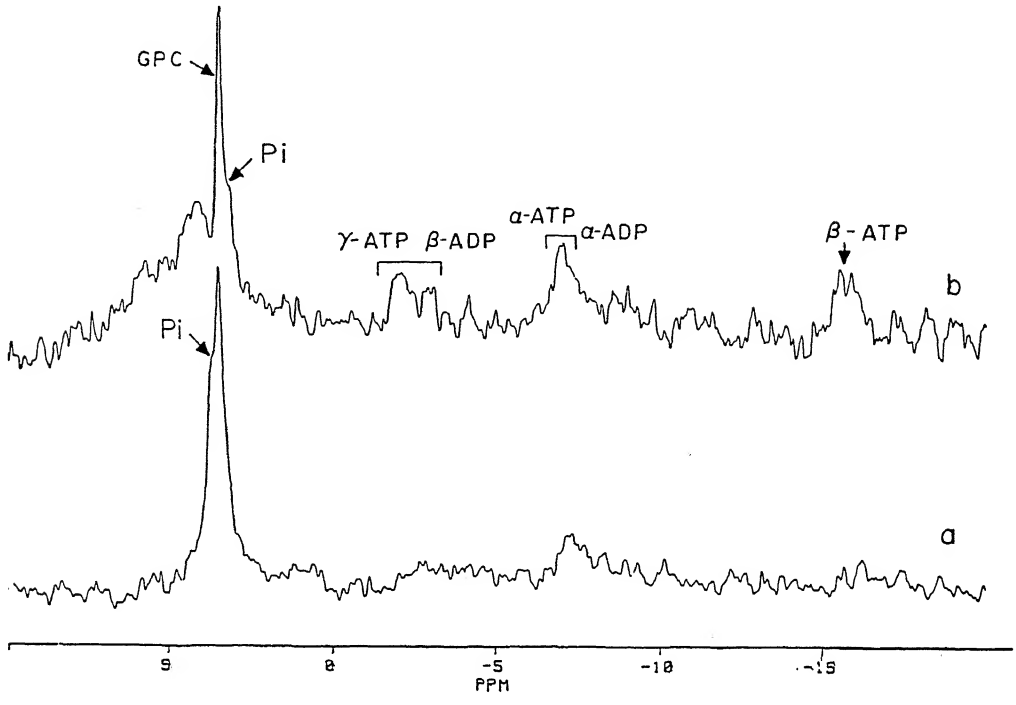

Fig. 3–^{31}P NMR is an important indicator of the ability of a living system to produce energy rich compounds such as ATP, GPC etc. The position of ^{31}P signal of inorganic phosphate is pH dependent and has been used to monitor *in-vivo* pH changes. The changes in this figure show the activation of starved spermatozoa (lower spectrum) after thawing and feeding the same with glucose (top).

starved cells are fed glucose, the ATP level builds up. Dead cells do not consume Glc and there is no increase in ATP/ADP ratio. Such cells also do not show motility or fertilizing capability. The position of P_i signal serves as an easy way to monitor the pH changes and hence the chemical activity of cells. The inorganic P_i exists in the cells in the form of $H_2PO_4^-$ or HPO_4^{--} depending on the pH and the NMR signals is at the position which is the time average of the chemical shifts of the various forms of the tri-basic acid. The measurement of pH in this way is a quick check on the metabolic activity of the cell.

Biochemical changes during cell maturation

The changes in the biochemical constituents of spermatozoa have been analyzed. When the cells are just born in caput region, one sees mainly ADP signal with very little ATP. ATP levels increase as the cells mature. There are also changes in the levels of several compounds during cell maturation as spermatozoa move from caput to cauda region. The total inositol (m-inositol+s-inositol) decreases with the cell maturation, while the relative concentration

of GPC and amino acids Arg, Glu and Gln increases. The signal for the unusual amino acid hypotaurine, which is an important compound for sperm survival capacitation and fertilization, is very weak in caput region. The amount of lactate, creatine and phosphocreatine is much less in cauda cells compared to caput while that of Glu, Gln, GPC and hypotaurine is higher (Fig. 2).

Effect of Arg on activity of spermatozoa

I will use this as an example of how we can learn about effect of certain compounds on metabolic pathways, which is of potential value in drug discovery and in study of inhibition of cell growth. L-Arg is an important molecule in sperm metabolism and is known to increase spermatogenesis. Administration of Arg to oligospermic and asthenospermic patients leads to both an improvement in sperm count and in motility. NMR studies show that the presence of Arg increases Glc and Fru consumption by cells with a concomitant increase of Lac production and decrease of pH. It also reduces damage due to the presence of ionizing radiation and reverses the impairment caused by glycolytic inhibitors (potential contraceptives). The influence of Arg on membrane lipid peroxidation induced by UV radiation, freezing and oxidizing agent have been studied. Irrespective of the nature of induction of peroxidation, L-Arg is found to reduce the extent of lipid peroxidation in a concentration dependent manner. It is also observed that both L-Arg and α-tocopherol act synergistically in preventing lipid peroxidation. Arg has a high degree of specificity in its catalytic activity as structurally related amino acids such as L-ornithine, L-Lysine and nitroarginine do not act as activators. NMR studies have also helped in establishing that Arg stimulates the nitric oxide synthesis in spermatozoa, which is a key factor in the enhancement of metabolic activity and also plays a role in preventing lipid peroxidation. It has been concluded that the mechanism of action of Arg is primarily through increased biosynthesis of nitric oxide in spermatozoa.

Use of solid-state NMR techniques for studying tissues

While cells and body fluids give relatively sharp and resolved NMR signals, spectral lines in conventional 2D NMR from tissues and stool are broad. However, these materials are of great importance for pathological analysis. The techniques of magic angle spinning and cross polarization helps in obtaining high resolution NMR spectrum of such solid like materials. This in turn has been applied to look for metabolic markers associated with various toxins, which have different organs as target. Griffin et al. for example, have studied rat testicular tissue and obtained spectra comparable to those obtained in cell suspensions. The technique can be applied with advantage to study membrane bound metabolites. This field along with NMR microscopy is in a development stage and is an area where rich dividends are expected.

Metabolism in whole organs

The use of volume selected localized spectroscopy in whole organs suffers from very broad lines and low sensitivity. It may be recalled that the cells in organs are fixed in space; additional broadening arises because of techniques in volume selection. Due to these reasons one has to select fairly large volumes (present limit is 2 cm cube).

Quantification is a major problem and only some of the most intense signals can be recorded. For example, water suppressed ^{1}H NMR of typically 1 mm^3 regions of brain shows signals of N-acetyl aspartate (which is a neuronal marker, and is indicative of healthy neurons), GPC, Lac and some amino acids. The levels of several of these molecules change with age and during diseases. The brain activity is often measured by looking at the paramagnetic effect due to oxygen levels in blood (BOLD), which in turn affects the intensity of water signal. NMR methodologies for metabolic studies in organs have seen remarkable progress in recent years and within its limitations, such studies are finding increasing applications. One remarkable example is the measurement of concentration of gamma-aminobutyric acid (GABA), using ^{13}C labeling of its precursor Glutamine. GABA is a major inhibitory neurotransmitter in mammalian central nervous systems (CNS). Occipital cortical GABA has been shown to be lower in patients with epilepsy, depressive disorders and its level are responsive to anti-epileptic drugs. Studies show that increased CNS levels of GABA is beneficial in treatment of epilepsy and other neurological drugs. One can focus at a small volume of interest and look at differences in the signal intensities of some key compounds such as ATP, PCr, lactate etc. by MRS. Using this information, one can diagnose diseases, differentiate healthy regions from diseased ones and follow chemical changes after surgery or during treatment. It is even possible to detect changes when a living being is doing a particular function such as motor movement or during learning (fMRI). In this respect, NMR has evolved as a serious competitor of positron emission tomography.

Applications of MRI and MRS in drug discovery and testing are rewarding areas of research. The techniques are used for measuring concentration, distribution and pharmaco-kinetics of drugs *in vivo* in human and animals and to assess if a new compound is exerting the same pharmacological effects in humans as in animals. Such studies can guide dose selection for the extensive and expensive clinical efficacy trials. In recent years, it has become possible to do metabolic studies in intact organs. For example, use of ^{19}F NMR spectroscopy to study the metabolism and pharmaco-kinetics of 5-fluorouracil in human liver and the pharmaco-kinetics of Lithium uptake in human brain/muscle by ^{7}Li NMR. It is useful for understanding the interaction of drugs at the receptor site, their activity and metabolism and in the design of drugs. Being non-invasive in nature, MRI and MRS can be applied to pre-clinical as well as clinical research as it allows repetitive measurements in same animal/subject, which is advantageous from economical and animal welfare point of view.

Conclusions

It is clear that the rapidly advancing techniques in high resolution NMR in solution and solid state have opened the field of studying metabolism in body fluids, cells and tissues. Such studies also complement whole body studies by providing more complete assignments and better differentiation between healthy and diseased cells. New metabolic pathways continue to be discovered. These studies also help in developments of molecular cloning of labeled proteins and nucleic acids, which are important for macromolecular structure determination. However, the most important contribution of such

studies may be in drug discovery, studies of drug metabolism and toxicology. With the emergence of proteomics and genomics, the role of linking metabolic differences in organisms cannot be over-emphasized. Three of the major NMR nuclei ^{1}H, ^{13}C and ^{31}P have been very effectively used for this purpose, while ^{15}N holds promise for studies involving amino acids. This has given a new dimension, which goes beyond genomics and proteomics, and is possibly a new frontier for NMR research. As mentioned, the main limitation of NMR is its relatively low sensitivity (detection limit of mM). However, the sensitivity in NMR has been going up every year and with this its power to open up new horizons in biological research. For the benefit of those interested in more details about this area, I have given a number of references with their titles.

Acknowledgements

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Engines at molecular scales

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Abstract

In recent literature there has been a lot of interest in the phenomena of noise induced transport in the absence of an average bias occurring in spatially periodic systems far from equilibrium. One of the main motivations in this area is to understand the mechanism behind the operation of biological motors at molecular scale. These molecular motors convert chemical energy available during the hydrolysis of ATP into mechanical motion to transport cargo and vesicles in living cells with very high reliability, adaptability and efficiency in a very noisy environment. The basic principle behind such a motion, namely the Brownian ratchet principle, has applications in nanotechnology as novel nanoparticle separation devices. Also, the mechanism of ratchet operation finds applications in game theory. Here, we briefly focus on the physical concepts underlying the constructive role of noise in assisting transport at a molecular level. The nature of particle currents, the energetic efficiency of these motors, the entropy production in these systems and the phenomenon of resonance/coherence are discussed.

(Keywords : ratchets/ molecular motors/ energetics/ transport coherence).

I. Introduction

Noise or fluctuations, that arise either due to the coupling of the system with external incompletely described system or from the bath is traditionally thought of as an unwanted effect. Recently, much work has been done the outcome of which reveal clearly the constructive role of noise in many systems[1-7]. Particularly, biological systems provide an important motivation to study the physics of active processes. In the molecular scale, these systems transduce chemical energy obtained from chemical reactions out of equilibrium into mechanical work, generating net motion in a very noisy environment. In terms of magnitude, the particle is acted upon by a noise power of about 8-9 orders of magnitude greater than the chemical power available to drive the motion. Even then the molecular motors, for instance, are able to move and transfer cargo from one point to another and sometimes against the potential gradient. They perform this useful work with high efficiency and reliability even when the environmental conditions are changing all the time.

Examples of molecular motors include cytoskeletal motor proteins namely kinesin,

dynein, etc., which move on the microtubules. Also molecular pumps, for example, sodium or potassium pumps etc., maintain active transport across membranes against a concentration gradient. What distinguishes these machines from their macroscopic counterparts or heat engines is the fact that they operate in a highly viscous medium which is characterised by low Reynolds number and are subjected to strong thermal fluctuations due to which their motion is stochastic. Hence these motors are termed as Brownian motors or rectifiers. Also, they operate at isothermal conditions. They work by harnessing the force of random motion in the surrounding medium in the absence of a conventional energy source and use it for creating directed motion. Here we focus mainly on some general physical principles behind such phenomena without going into the details of its biological implications.

Any system which is in equilibrium with a thermal bath at temperature T has the presence of noise in it. Though these thermal noise/fluctuations are ubiquitous, the validity of the second law of thermodynamics forbids the harnessing of noise for useful purposes without spending any energy from the external sources. A Brownian particle executes a random motion in a liquid without any preferential direction. The principle of detailed balance, which essentially means that the rate of forward motion is equal to the rate in the backward direction, forbids current in any preferential direction and hence one cannot extract useful work. In other words, one can extract energy only when the system is driven away from equilibrium. This has been very well demonstrated by Feynman in his 'Feynman Lectures on Physics[8] by introducing a mechanical ratchet and pawl subjected to thermal fluctuations to demonstrate the impossibility of the violation of the second law of thermodynamics. Hence building a motor that uses thermal energy from a single heat bath to do mechanical work is not possible.

II. Conditions for the effect

The model to understand such noise induced active transport in a fluctuating environment is provided by the so called Brownian ratchets. These are systems with an underlying spatially asymmetric periodic potential, that exploit the nonequilibrium fluctuations, present in the medium, to generate a directed flow. The effect of the thermal environment is modeled by considering randomly fluctuating force $\xi(t)$ and a concomitant viscous (frictional) force with a friction coefficient η. η and random noise $\xi(t)$ with $< \xi(t) >= 0$ are related through the fluctuation-dissipation theorem, i.e., $< \xi(t)\xi(s) > = 2\eta k_B T\delta(t-s)$. Physical models like flashing ratchets, rocking ratchets, time asymmetric ratchets, inhomogeneous (frictional) ratchets etc., have been proposed to achieve essential nonequilibrium conditions for net motion in a periodic system. As long as the system is left alone and remains in thermodynamic equilibrium particles in a ratchet cannot diffuse in any preferential direction, in spite of the spatial asymmetry in the potential. But in the presence of additive or multiplicative nonequilibrium fluctuations the particles in general start to move in one direction as the principle of detailed balance does not hold in this case. Thus both nonequilibrium fluctuations and spatial or temporal asymmetry in potential conspire to generate a unidirectional flow in the absence of bias. In the following we briefly discuss the essential ideas behind some of the ratchet models.

III. Different types of ratchets

A. Flashing ratchets

This is a simple model that closely resembles the mode of operation of protein motors. In this model the periodic potential is allowed to fluctuate with finite time correlation between two states characterised by different barrier heights. For example, the overdamped Brownian particles are subjected to two potential states periodically, i.e., V_{on} which corresponds to an asymmetric saw tooth like potential for a time τ_{on} and V_{off} which corresponds to zero potential (flat) state for a time interval τ_{off} as is shown in Fig. 1. During the period when the potential is *on* the particles will slide down the potential slope to the bottom of the potential minima due to which there is a peak in the probability density of particles at these minima. Switching the potential *off* allows the particles to diffuse freely and the density of particles spread into a Gaussian curve centered around the minima as shown in Fig. 1 b. At the end of τ_{off} the potential is again put back to the *on* state for an interval τ_{on} and the particles will again slide down along the direction of local force to the nearest minima, Fig. 1c. This process is continued indefinitely resulting in a net current in one direction because of spatial asymmetry in the potential within a period.

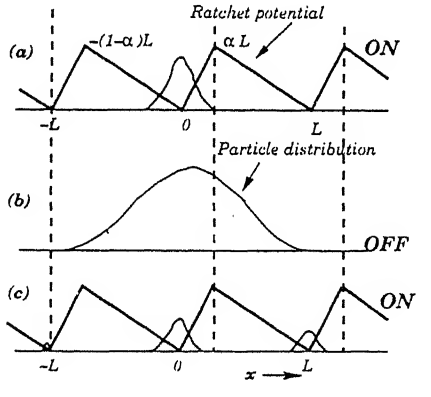

Fig. 1–Flashing ratchet model. Here L is the period of the potential and α is the asymmetry parameter.

The main point to be taken care of is the time interval between switching *on* and *off* periods of the potential. If τ_{off} is adjusted such that by the end of τ_{off} the diffusive motion takes the particle out of the earlier existing potential minima in the steeper slope (smaller distance) direction of the saw tooth potential but fails to do so in as much proportion in the gentler slope (larger distance) direction, then in the next *on* interval of the potential the particles will slide along the gentler slope to the adjacent minimum of the saw tooth potential. This process of sequential flipping of the potential between *on* and *off* states is continued and in the long time limit one gets a net flow of current to the right side. The system is supplied with the required energy to flip the potential states externally thereby rendering the system nonequilibrium. It is to be emphasized that thermal fluctuations are necessary for the working of flashing ratchets. Moreover, no macroscopic bias is applied to the system.

1. Equivalence to Carnot engine

The flashing ratchet model where the potential is flipped between *on* and *off* states is analogous to the case where the particle is coupled to two temperature baths. From statistical mechanics it is well known that the probability to cross a barrier is governed by the factor exp $-(V/k_BT)$ where V is the potential barrier height. When the temperature T is large the average kinetic energy of the particle is large and so is the violent thermal fluctuations. Due to this the particle hardly feels the presence of potential in comparison with the thermal noise and

hence the probability to cross the barriers is large. This is equivalent to the particle being in the *off* state of the flashing ratchet discussed above. In the opposite case when the temperature of the medium is small, the average kinetic energy and the thermal fluctuations are small and the particles will feel the presence of the asymmetric potential. This case is akin to the *on* state in the flashing ratchet. Thus the systematic coupling of the particle randomly to two temperature baths is equivalent to flashing ratchet model where the potential is switched *on* and *off* and in the long time limit one gets unidirectional current. In this spirit the ratchet system has direct equivalence to a Carnot engine which extracts work by making use of two thermal baths held at different temperatures.

The relevant system variables for the ratchets are temperature $T(t)$ and the position $x(t)$ while that for a Carnot engine are pressure and volume. However, there exists qualitative differences between the two. For the case of ratchets, after one periodic variation in time of $T(t)$ or one temperature cycle the particle may or maynot come to the same position or in other words there is no synchronization between the relevant system variables. But for a Carnot engine there is a complete synchronization between the relevant system variables along the cycle. The ideal Carnot engine moreover will not be simultaneously in contact with two temperature baths assuring the reversible mode of operation. In contrast, ratchets or molecular motors work in an intrinsically irreversible mode of operation with a very low efficiency. It has to be emphasized that Carnot engine gives high efficiency only in the quasi-static mode of operation and though there is net work done by the engine the net power delivered in the cycle is zero. For the case of molecular motors we may get a higher efficiency in the nonadiabatic regime (i.e., by increasing the frequency of oscillation or cycling) as compared to the values obtained in the adiabatic or quasi-static regime of operation. This behaviour is quite contrary to the case of reversible macroscopic heat engines. The distinguishing factor of a Brownian motor is that noise plays a dominant role and that noise may facilitate energy transduction leading to high efficiency of these molecular engines which is counterintuitive[9].

In these molecular engines noise and associated dispersion of particles are no longer thought of as a hindrance, but are instead incorporated as a part of the design. Moreover, an efficient microscopic engine is not necessarily the microscopic equivalent of an efficient macroscopic engine.

2. Current reversals and mass separation devices

With a judicious choice of asymmetric potential the current reverses its sign as a function of suitable system parameter. This phenomenon is called current reversal. Thus, Brownian particles with different friction coefficients, masses or charges move in opposite directions and hence they can be readily separated. This is a new modern method of separating particles at nanometer scale. To illustrate the phenomenon of current reversal consider a potential of the form $V_\lambda(x, t) = \lambda V_2(x, t) + (1-\lambda) V_1(x, t)$ where $V_1(x, t)$ and $V_2(x, t)$ are two fluctuating ratchet potentials such that the unidirectional flow of currents in $V_1(x,t)$ and $V_2(x, t)$ are in opposite directions in the presence of nonequilibrium fluctuations and A is a parameter between 0 and 1. If $V_2(x, t)$ is a mirror reflection of $V_1(x, t)$, the fluctuating ratchet potential shown in Fig.

1, then under the influence of $V_2(x, t)$ alone current flows in the negative direction. The current has a smooth dependence on mass and the higher the mass the lower is the current. A particle of mass m will move in respective directions depending on the potential to which it is subjected to. Suppose that the particle is subjected to the potential $V_\lambda(x)$ which is a combination of $V_1(x, t)$ and $V_2(x, t)$. The plausible curve for unidirectional current as a function of λ in shown Fig. 2. When λ is zero there will be contribution only from $V_1(x, t)$ and one gets a current which is positive. When λ = 1 the contribution to the current will be from $V_2(x, t)$ and hence the flow will be in the opposite direction. Thus by continuously deforming one potential into another, i.e., $V_1(x, t)$ into $V_2(x, t)$ there must exist an intermediate potential with the property that the particle current is zero at some finite value of the parameter λ_c. Hence there is a critical λ_c at which the current curve passes with a finite slope through this zero point thereby implying the existence of current inversion as a function of λ.

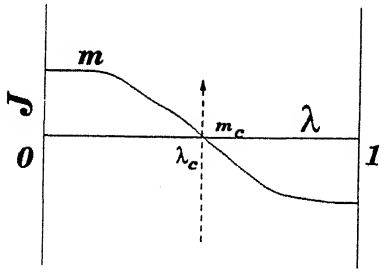

Fig. 2– Illustrates current reversal.

Once a current inversion upon variation of one parameter of the model is established an inversion upon variation of any other parameter can be inferred along the same line of reasoning. Suppose we fix a point, say, the value of $\lambda = \lambda_c$ at which the current for a particular mass, m_c, is zero. Now if we vary the mass around the value m_c one would see that the current as a function of mass will smoothly go through this zero point at $m = m_c$ with a finite slope. This means that a current reversal is obtained as a function of mass. That is, particles of mass greater than m_c and that with mass less than m_c get separated in opposite directions.

The phenomenon of current reversal can play a major role in separation devices. This method of particle separation have many features far superior than the existing methods like electrophoresis, centrifugation, chromatography etc., which rely on the motion caused by long range gradients. In these methods the thermal noise in turn degrades the quality of separation due to the diffusive broadening of the bands. It is also possible to get multiple current reversals by properly choosing the potential as a function of system parameters. By multiple reversals one can separate blocks of particles of different masses with parameters within a characteristic window. It may be noted that for two dimensional ratchets, particles of different masses get separated in different directions.

3. Parrondo's paradox

The concept of Brownian ratchets, where there is rectification of fluctuations to give unidirectional current also has its extensions to game theory opening up a new area of paradoxical gambling games under the subject of Parrondo's paradoxes[10]. These games can be thought of as a discrete time version of flashing Brownian ratchets with the interesting consequence that by randomly switching between two losing games one tends to win. To begin with, consider a flashing ratchet model in the absence of any external force as discussed

in Sec. III A with a current flow towards the right, Fig. 1, or in the positive direction when the potential is fluctuating between the *on* and *off* states. To this a constant tilting force F, as in Fig. 3, is applied opposite to the direction of current in the ratchet. Then, as expected, the current (J) in the positive direction decreases and beyond a particular value called the stopping force F_c the current crosses over to the negative direction. This is illustrated in the J *vs.* F plot in Fig. 3. Let us consider a particular value of force say F_A in the J *vs.* F plot. The corresponding flashing ratchet profile is as in the figure. In the presence of a bias force F_A, the current flows in the negative direction as expected in both the *on* and *off* states of the potential in the absence of flipping. But flipping between the *on* and *off* states randomly for a long time will result in a current in the positive direction. Thus at the point F_A one gets current in the positive direction though when considered separately (i.e., when the potential is as shown either in Fig. 3a or in Fig. 3b) the flow of current is in the negative direction.

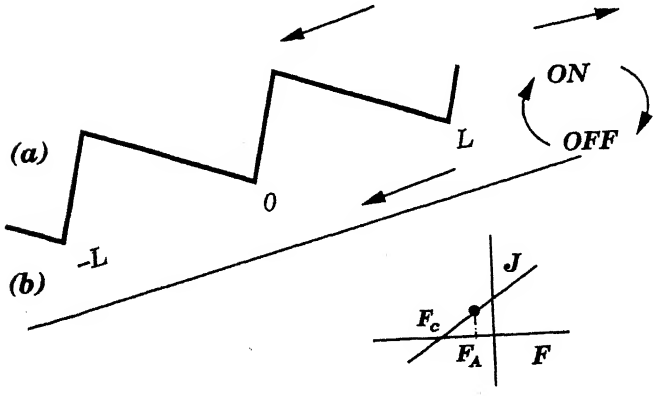

Fig. 3– Illustration of Parrando's paradox. (a) and (b) correspond to the *on* and *off* potential states in the presence of bias field.

The current in the negative direction for the two different potentials is analogous to a losing game and thus alternating randomly between these two losing games one has a finite probability to win (current in the positive direction). The game reveals the fact that the outcome of the alternation of two stochastic dynamics can significantly be different from each separate one. Parrondo's paradox, where one basically converts two losing strategies into a winning one, has numerous applications in the field of economics, sociology and many other interdisciplinary areas. An example is the formation of spatial patterns in spatially extended systems by alternation between two dynamics which in turn is absent in the presence of only a single dynamics.

B. Rocking ratchets

In the ratchet model we had considered before namely, flashing ratchet, the potential fluctuates between *on* and *off* states. Another type of ratchet corresponds to rocking ratchets where given an asymmetric potential one applies a random time varying force with mean zero. Due to the anisotropy of the potential (a special case is shown in Fig. 4a), when a force having same magnitude but different signs + F and - F are applied, the motion of the particle on the average will be along positive and negative direction respectively. However, particles will have to overcome only the smaller barriers along the direction of their average motion in the presence of a positive force as opposed to the case when the force is negative with the same magnitude. We consider a case where the average slope of the saw tooth potential, Fig. 4a, is changed in time either slowly or abruptly with a finite maximum value on either side of the zero slope line ensuring that the time average of the force acting on the particle due to rocking is zero (Fig. 4b and c). The rocking or changing of slopes

can be done either periodically or randomly in time.

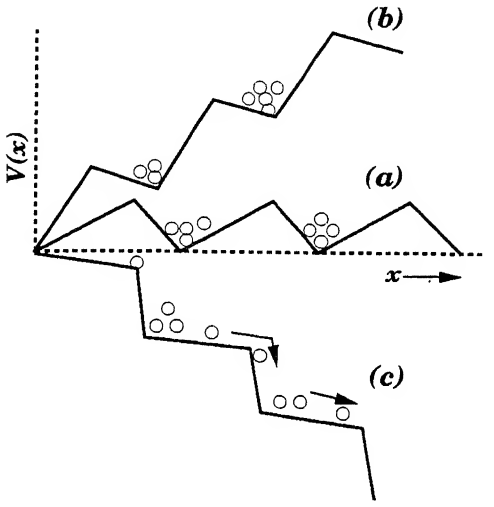

Fig. 4 – Rocking ratchet model.

At very low temperature when the particles do not have enough energy to overcome the barrier the particles get trapped in the minima of the potential. A special case of rocking force, + F and - F, imposed on the ratchet potential is shown in Fig. (4b and 4c) respectively. For the case when force is negative, Fig. 4b, the particle remains trapped in one of the trenches whereas when the force is positive, Fig. 4c, the particle is capable of running down the potential hill. With such a geometrical construction one can notice that the current when the force is $+|F|$ is not equal and opposite to the case when force is $-|F|$. In otherwords, $J(|F|) \neq J(-|F|)$ remains valid even for finite temperatures. Thus system acts as a nonlinear rectifier in the presence of zero average periodic or random force.

Unlike in the case of flashing ratchets, the direction of current for the rocking ratchet is in the direction of the steeper slope and this mechanism of rocking is equivalent to generating dc current in semiconductor pn junctions under an applied ac bias.

C. Inhomogeneous ratchets

There is yet another type of ratchet, namely, frictional ratchets[7,11] which unlike the ones discussed above gives unidirectional current even in the presence of spatially periodic symmetric potential, $V(x)$ but in the presence of space dependent diffusion coefficient $D(x)=k_BT(x)/\eta(x)$. The space dependence of diffusion coefficient could arise either due to space dependent temperature $T(x)$ or space dependent friction coefficient $\eta(x)$. Such inhomogeneous systems are common in nature. For example, particles diffusing close to surface have space dependent friction coefficient. The molecular motor proteins are believed to be moving close along the microtubules and therefore experience space dependent mobility. Semiconductor systems and superlattice structures also have space dependent friction coefficient. The peculiarity of this type of ratchets is that the system dissipates energy during its time evolution differently at different places due to the space dependent diffusion coefficient arising from variation in temperature $T(x)$ which in turn implies that the system is out of equilibrium. The only criterion that has to be satisfied here is that both the potential $V(x)$ as well as temperature $T(x)$ has to be periodic and should be separated by a phase difference other than 0 and π with respect to the potential as shown in Fig. 5. A similar effect of variation in temperature can also be obtained if we have a medium with space dependent friction coefficient $\eta(x)$ in the presence of external noise. The noise being externally imposed the system always absorbs energy due to the absence of a concomitant loss factor. At the regions

where the friction coefficient is high the overdamped particle stays for a longer time due to which the possibility of absorption of energy from the external noise in that region is correspondingly high. This leads to an increase in the local temperature in these regions. Thus system with space dependent friction at constant temperature in the presence of external parametric noise is equivalent to a system with a space dependent temperature field.

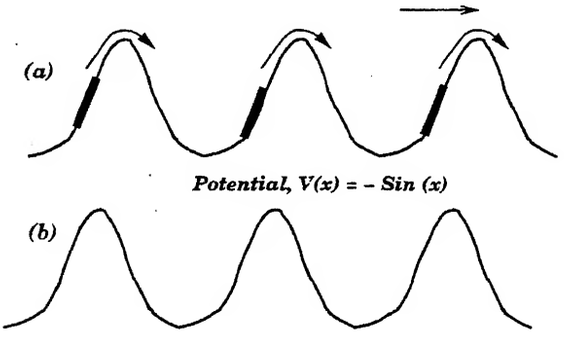

Fig. 5 – Illustration of an inhomogeneous ratchet. The temperature and potential profiles are depicted in the figure.

To illustrate the net unidirectional transport in these systems consider a periodic potential $V(x)$ as shown in Fig. 5a. Also consider a space dependent temperature profile with same periodicity as that of potential but shifted by a phase difference ϕ as shown in Fig. 5b. In Fig. 5a the darkened regions specify regions of higher temperature corresponding to the regions where the temperature profile has peaks Fig. 5b. The particle in the darkened regions (high temperature regions) on the average gains more energy as compared to other regions. As a consequence the particle in any potential minima will find it easier to cross the peak of the potential and go over to the right side than to the left side. Hence current in the right side is assured. The magnitude as well as the direction of current depends on the phase difference ϕ.

It may be noted that unidirectional motion in inhomogeneous systems arise as a corollary to the well known Landauer's blow torch principle[12]. This principle states that the behaviour of nonequilibrium systems will depend sensitively on the specific details of its kinetics, even on pathways that traverse infrequently occupied kinetic states far from the stable state. In otherwords, the stability criteria which examine only the immediate vicinity of a locally stable state are inadequate to assess the relative stability of states in nonequilibrium systems. In contrast, microscopically reversible systems can well be characterised by criteria that depend only on the local neighbourhood of the equilibrium state.

IV. Energetics of Brownian motors

As mentioned above, ratchets extract energy from random fluctuations and generate currents or ordered motion. In this sense they can be considered as information engines analogous to Maxwell's demon which extract work out of bath at the expense of an overall increase in entropy. The usefulness of any engine lie in the extent of work that can be efficiently extracted out of it. The molecular motors in living cells are found to be very efficient in their noisy environment. In all the ratchet models that we have discussed above no useful work has been performed. This is because particles moving in a periodic potential system ends up with the same potential energy even after crossing over the adjacent potential minimum. There is no extra energy stored in the particle which can be usefully expended when needed. To have an engine out of a ratchet it is

necessary to use its systematic motion to store potential energy which inturn is achieved if a ratchet lifts a load. Thus for the ratchet to perform work a small force called load (Fig. 6) has to be applied opposite to the direction of current in the ratchet. Then the particles will keep on moving, on the average, against the force or load performing work. Part of the input energy, E_{in}, coming from the source of nonequilibrium is converted into mechanical energy related to the load.

A general framework has been developed wherein the compatibility between the Langevin or Fokker-Planck formalisms, used to discuss stochastic processes, and the laws of thermodynamics, which characterize the thermal and mechanical behaviour of macroscopic systems, have been established[13]. The concept of heat on mesoscopic scales has been defined in terms of Langevin dynamics and the essential point behind this formalism is that the heat transferred to the system is nothing but the microscopic work done by both the frictional and random force in the Langevin equation (i.e., work done by the bath on the system). This is also consistent with the fact that we cannot control all the details of energy transfer which inturn leads to the concept of heat (*via* stochastic dynamics) as a form of energy flow. The subject of the energetics of Brownian motors has developed into an entire subfield on its own right. Fig. 6 represents the energy flow between the isothermal Brownian motor and its surroundings. The first and the second law of thermodynamics is now given as

$$E_{in} = Q + W \qquad (1)$$

$$\Delta S_{agent} + \frac{Q}{T} = S_{prod} \geq 0 \qquad (2)$$

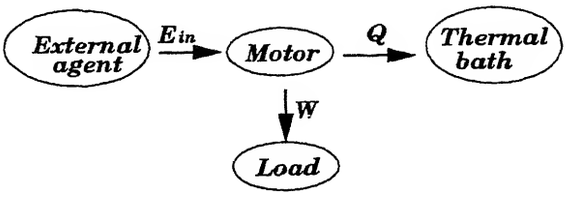

Fig. 6 – Schematic figure of the energy flow in a Brownian motor.

In the above equation E_{in} is the input energy into the system from the external agent, Q, the heat dissipated to the bath, and W the work done. All these quantities can be defined for each microscopic realization of the motion of Brownian particle or motor. Eqn. (1 and 2) correspond to the first and second law of thermodynamics. Again, ΔS_{agent} is the change in entropy of the external agent, Q/T is the entropy given to the bath and S_{prod} is the total entropy production of the universe. T corresponds to the absolute temperature. Magnitudes of all the physical quantities are taken over a cycle or per unit time in the stationary regime. In this regime the entropy and the internal energy of the motor (system) which are the state variables does not change. The formal expressions for all the above mentioned physical quantities are known in terms of the probability distribution of the particles.

Using this framework of stochastic energetics one can readily calculate various physical quantities like efficiency of energy conversion ($\eta = W/E_{in}$), energy dissipation (hysteresis loss), entropy production[14], input energy, work etc. The important point to be noted here is that an analysis of fluctuations, which is completely ignored in the working of heat engines at larger scale,

is essential for the calculation of efficiency of ratchet systems at the molecular scale. The efficiency of the Brownian motors is sensitively dependent on system parameters.

The study of the efficiency of energy transduction by different types of ratchet models show the ratchets to have very low efficiency. The observed efficiency values of the several ratchet models like flashing ratchets, rocking ratchets etc., are found to fall in the subpercentage regime (< .01). This is due to the fact that every time the potential changes the particle distribution also changes and tries to adapt to the changing environmental conditions. This leads to an inevitable loss in the medium or in other words the mode of operation of the ratchets is intrinsically irreversible. As a consequence the unattainability of Carnot efficiency in Brownian heat engines has been emphasized in literature. Currently, the notion of reversible ratchets where the energy dissipation or entropy production is almost zero are being pursued. These reversible ratchets are sometimes termed as adiabatic pumps wherein transport of particles with zero entropy production is generated by cyclic adiabatic variations of atleast two parameters of the periodic potential (which are out of phase in time) in the absence of bias[13].

The energetic efficiency of a ratchet is not an intrinsic property of the device and it depends on the characteristics of the imposed external load. By a judicial choice of the external load one can improve the efficiency considerably. Recently[15] a flashing rachet model has been developed, wherein with two asymmetric doublewell periodic-potential-states displaced by half a period a high efficiency has been achieved due to the blocking of particle motion in the direction opposite to that of the net average current. Such flashing ratchet models were found to be highly efficient with efficiency an order of magnitude higher than in the earlier models. The basic idea behind this enhanced efficiency is that even for diffusive Brownian motion the choice of appropriate potential profile ensures suppression of backward motion and hence a reduction in the accompanying dissipation.

We have studied the motion of a particle in a new class of rocking ratchets rocked purposefully as to favour current in one direction but to suppress motion in the opposite direction. This is accomplished by applying a temporally asymmetric but unbiased periodic forcings. It may be noted that in this type of ratchets a larger force field is applied for a short time interval of the period in the forward direction as compared to a smaller force for a longer time interval in the other direction. The intervals are so chosen that the net external force or bias acting on the particle over a period is zero. In these new class of temporally asymmetric driven ratchets one gets unidirectional current even in the presence of spatially symmetric potential[16].

At low temperatures when k_BT is much less than V_o, the modulation amplitude of the periodic symmetric potential, significant current arises only when the bias field is greater than a critical field F_c, the value of which should be greater than V_o[17]. If the bias field is less than V_o, the particle will feel the barriers and hence current flux in the negative direction is very small or there is blocking of current. A significant current flux in the positive direction arises only when the temporally asymmetric bias force field in that direction is greater than V_o. When this condition, of bias field being less than V_o in the backward direction and

being greater than V_o in the forward direction, is satisfied the barriers for motion in the forward direction disappears and one gets unidirectional current. Interestingly, such choice of forcings help in obtaining rectified currents 'with high efficiency of the order of 50% without fine tuning of the physical parameters. This efficiency is several orders of magnitude larger than the obtained efficiency in several other ratchet models. Moreover, the range of parameters of operation of such ratchets is quite wide sustaining large loads.

A. Currents, Stochastic resonance and Coherence

The noise induced currents in most of the ratchet systems exhibits a peak with respect to noise strength and other physical parameters. Such peaking behaviour is expected when system exhibits a resonance phenomena. Infact, some recent studies have tried to reveal the relation between two unrelated phenomena, namely stochastic resonance and Brownian ratchets in a formal way through the consideration of Fokker-Planck equations. We have analysed this issue by using the method of stochastic energetics in several classes of adiabatically rocked ratchets.

The resonance behaviour can be well characterised by the behaviour of input energy. It is expected that at resonance the system extracts maximum energy from the external source and hence in the time periodic stationary state this energy is dissipated to the environment (hysteresis loss). Our studies show the input energy to have a monotonous behaviour as a function of noise strength. Thus the resonance like feature observed in the nature of currents as a function of noise strength is not related to the intrinsic resonance in the dynamics of the particle with the external ac drive. The above observations are valid only for a class of adiabatically rocked ratchets.

The presence of net currents (ordered motion) in the ratchets increases the amount of known information about the system than otherwise. This extra bit of information comes from the negentropy or the physical information supplied by the external nonequilibrium bath. The amount of information that is transferred by the nonequilibrium bath is quantified in terms of algorithmic complexity of the position of Brownian particle. It has been argued that the algorithmic complexity or Kolmogorov information entropy is maximum when the current is maximum[18]. Since the currents are generated at the expense of entropy we naively expect the maxima in current to be related to the maxima in the over all entropy production as a function of noise strength. However, we have shown that the total entropy production does not extremize at the same parameter value at which the current exhibits a maximum[14]. The fact that noise strength at which the maxima seen in both current and entropy production do not coincide may be related to the quality of the current or the coherence in transport. Noise induced currents are always accompanied by a dispersion or diffusion. When the diffusion is large, $\Delta x >> L$ with Δx being the diffusive spread when the mean position of the particle is shifted by a distance L which is the period of the potential, then the quality of transport degrades and the coherence in the unidirectional motion is lost. The coherent transport (optimal transport) refers to the case of large mean velocity at fairly small diffusion (see Fig. 7). This is in turn quantified by a dimensionless Peclet number which is the ratio of current to the diffusion constant[19]. From Fig. 7 we see that in both the cases there is a noise

induced current of the same magnitude, but transport in Fig. 7a is more coherent due to the fact that particles are reliably transferred from one point to the other due to the less diffusive spread. In Fig. 7b though there is a shift in the peak (or there is current of same magnitude) due to the large diffusive spread the probability that the particle is delivered to the desired region is less. There is a finite probability that the particle may still be around the region where it has started.

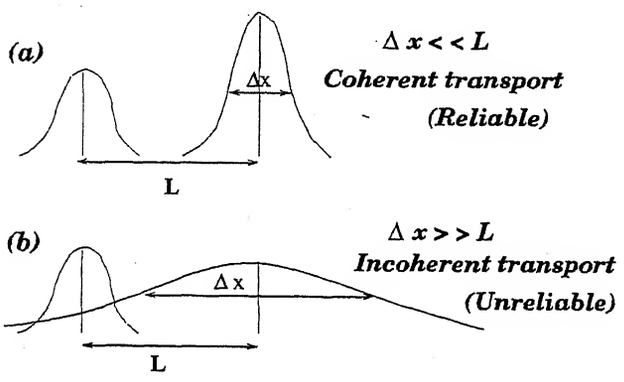

Fig. 7– Evolution of particle distribution for a given time interval is depicted for two separate cases of particle transport.

For a given magnitude of current the transport may be coherent or incoherent. Thus analysis of the relation between current and the entropy production requires not only the magnitude of current but also the quality of transport. These studies are expected to reveal a deep connection between efficiency, quality of transport, entropy and information.

V. Conclusions

To summarize, we have given a qualitative picture of the constructive role of noise in nonequilibrium systems. This area has attracted great interest from diverse areas of science and technology. We have presented a brief description of the different ratchet models or Brownian motors and also the method of stochastic energetics that was developed in order to understand the energetics in such systems with special emphasis on our work and results.

In our discussion so far we had restricted only to the case of isolated Brownian motors. Infact, the cooperativity among Brownian motors has far reaching consequences[3]. The coupling among these motors can lead to a marked increase in the efficiency of energy transduction as well as the magnitude of macroscopic current. Cooperative motors also exhibit other fascinating phenomena such as phase transitions, normal to anomalous hysteretic behaviour, absolute negative mobility etc. The parallel development in mesoscopic systems has led to the discovery of quantum ratchets. These quantum ratchets make use of quantum effects such as tunneling and wave interference effects. Such electron ratchets can not only be used to generate particle current but also to pump heat in the reversible mode of operation.

To conclude, we have shown that molecular motors work as engines at molecular scale. These microscopic engines are not the microscopic equivalent of the efficient macroscopic engines that we come across in our daily life. Noise play an inherent constructive role in the mode of operation of these engines. Thus noise is not a nuisance but rather an inseparable part of the design of the engine operation. In some cases increase in noise strength is found to even enhance the efficiency of these engines. The operation of these engines are done by the engines themselves and they are out of equilibrium. As such there are no general principles that determines the mode of optimal efficiency

of these engines. Further studies in this interdisciplinary area could lead to a better knowledge of the functioning of these biological motors in living cells and also in the creation of efficient man made nanoscale machines. Such studies could also bear important consequences in understanding the fundamental issues in none-quilibrium statistical machnics.

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Docking strategies in drug design and it applications to HIV-1 Tat protein inhibitors

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Abstract

Structure-based drug design involves the construction of ligands that fit into the active site of a receptor, forming favourable interactions with residues in the active site. Molecular docking procedures are central to all structure based drug design strategies. In this review, we cover the various approaches employed in molecular docking and conclude by showing how docking studies have helped to elucidate the putative binding regions, the binding affinities as well as binding modes for both the *D*- and *L*- forms of some HIV-1 Tat protein inhibitors – penicillamine (Pen), 2,3-dimercapto-1-propanol (DMP) and N-(2-mercaptopropionyl)-glycine (MPG).

(Keywords : docking strategies/HIV-1 Tat/tat inhibitors).

Introduction

Advances in molecular biology and protein biochemistry have made it possible to get reasonable quantities of purified proteins. The three dimensional (3D) structure of these proteins can be solved either by X-ray or multi-dimensional nuclear magnetic resonance (NMR) techniques. For those proteins whose structure cannot be obtained by an experimental method, homology modeling (Comparative Modeling) allows us to predict their 3D structures. Structure-based drug design uses this structural information to design new drugs with better properties like higher affinity and decreased side effects. Central to most structure-based drug design techniques is molecular docking, whereby ligands are positioned in the active site by one or more techniques. We first give an account of the principles behind the various docking methodologies and then present our work on the application of docking strategies to elucidate the putative binding regions, the binding affinities as well as binding modes for both the *D*- and *L*- forms of some HIV-1 Tat inhibitors – penicillamine (Pen), 2,3-dimercapto-1-propanol (DMP) and N-(2-mercaptopropionyl)-glycine (MPG).

Docking Strategies

Interactive Molecular Graphics

With interactive graphics the user with the help of the mouse places, rotates and

translates the ligand within the active site and also additionally rotates selected torsions within the ligand. This 'crude' docked structure is then subjected to energy minimization (discussed below). The *Sculpt* program[1] is based on interactive molecular docking.

Docking by Energy Minimization

The substrate is placed within the "active site" either manually or by some computational tool and the system is then subjected to energy minimization. This method has been successfully applied to study the inhibition of HIV-1 reverse transcriptase (RT) by anti-AIDS drugs AZT and ddC[2]. In this process, the substrate slides down into a favorable niche in the enzyme pocket, which may be a local minimum on the Potential Energy Surface (PES). By changing the orientation and position of the substrate in the active site, and subjecting each one to a thorough energy minimization, one can investigate several other 'binding modes'. In this way, the 'correct' binding mode with the lowest energy has been achieved.

Docking by Superimposition

New ligands can be docked into the binding site by superimposing selected atoms of a ligand onto corresponding atoms of another ligand already positioned in the active site. This method will work well if the two ligands are quite similar. Alternatively one can use atom or 'target' points in the active site to position the ligand. One of the earliest and also the most widely used of such methods is the program *DOCK*. First target points or "spheres" for superimposition are generated from the molecular surface of the enzyme. The program superimposes at least four ligand atoms onto four target points. If there are m heavy atoms in the ligand and n target points or spheres in the binding site, then there are m x n different ways to match the first atom with the first target point. After the first pair has been matched there are (m-1)(n-1) remaining ways of making the second pair and (m-2)(n-2) ways of making the third pair and so on. For a small ligand with 10 to 20 heavy atoms and a binding site generally represented by 20 to 60 target points, the total number of 4 pair matchings is in the billions. However, the vast majority of these matchings are geometrically impossible. For *e.g.* if the distance between the first and second atoms is 3 Å, then they cannot be simultaneously matched with target points that are 15 Å apart. The many superimpositions determined by *DOCK* are then scored according to shape complementarity.

In the 'Directed *DOCK*' superimposition, both shape complementarity as well as hydrogen bonding is used to find positions and orientations for the ligand in the binding pocket[4]. There are two target points: one the conventional 'sphere centers' and the second points are positions a ligand atom would occupy if it made an idealized H-bond with the protein. The algorithm then pairs ligand atoms with conventional sphere centers and it also pairs ligand heteroatoms with corresponding H-bond target points. Besides the original 'shape complementarity' scoring function, a force-field scoring function and a hydrophobicity function can also be used. There are several other programs like *CLIX, FLOG etc* which are based on the general theme of *DOCK*.

Docking Flexible Molecules by Superimposition

The superimposition paradigm described above works well with rigid molecules, but is not well suited for conformationally flexible ones. One way to do this for flexible molecules, would be to generate multiple conformations (say by Distance Geometry or Systematic Search) and then dock each conformation independently. In the approach of DesJarlais *et al*, the flexible molecule is divided into rigid fragments each of which is independently docked into the protein binding site and scored[5]. The high scoring fragment positions and orientations are recombined with energy minimization to obtain docked ligands. Leach and Kuntz identify a single rigid 'anchor' fragment, which is docked (Directed *DOCK*) into the binding site. Systematic search (SCS) is then used to identify low energy conformations for the flexible substituents, which are reattached to the anchor fragment in each of its previously determined positions[4]. Finally, all rotatable bonds in the fully constructed molecules are searched using SCS to find low energy binding modes for the whole molecule.

Docking based on Molecular Dynamics (MD)

Since MD simulates motion, it is possible to carry out a MD simulation where the ligand slowly diffuses into the active site of the protein, explores alternate conformations and binding modes and eventually chooses one particular binding mode. One advantage of MD simulations is that both solvation and protein flexibility can be taken into account. However, with present day technology, MD simulations are limited to time scales of a few ns. Ligand-protein binding requires microseconds or longer[6], and this time scale is well beyond the reach of routine MD calculations for many years to come. The binding process could be 'hastened' by introduction of an artificial restraining force between the ligand and a defined point in the active site[7], the force constant of which will determine how quickly the ligand descends into the binding pocket. MD simulations can also be used to generate alternate orientations and conformations in the binding pocket, which can then be refined by energy minimization. The breadth of the search in MD can be increased by adopting a variable simulation strategy where the translational motion of the ligand is simulated at a high temperature, the internal motions at room temperature and the motions of the receptor at a still lower temperature[8]. The local elevation method is another technique which can help to broaden the search by penalizing structures that have already been visited. Mixed Monte Carlo (MC)/MD methods with alternating cycles of MC and MD, where MC helps to jump over barriers and MD explores neighboring low-energy structures, can be gainfully used in docking studies[9].

Docking using Monte Carlo (MC) Principles

MC is used to randomly place the ligand in the active site, which is followed by energy minimization or MD to refine the interaction with the protein. This cycle is repeated a large number of times, until convergence is reached. The technique is good for small molecules, but inefficient for larger flexible ligands, because so much time is wasted on minimization of configurations that are trapped in high-energy states and secondly because many

of the successful minimizations converge to the same local minimum.

In the multiple copy simultaneous search (*MCSS*) method[10] 1,000 to 5,000 starting positions for the ligand are generated and then all structures are minimized simultaneously, where each copy of the ligand is allowed only to interact with the protein, but not with other copies. The program saves computer time by suspending the minimizations periodically (every 1,000 steps) to identify redundant minimizations that are converging to the same minimum. By allowing some degree of freedom for the protein, it is possible to construct multiple ligand trajectories in the presence of a single protein trajectory.

The above-discussed MC procedure does not learn from experience, and cannot focus on low energy structures. The Metropolis MC procedure generates a sequence of structures that can first locate and then explore low-energy regions of the conformational space. Its similarity to MD means that it has difficulty climbing barriers and can get 'stuck' in particular regions of conformational space. Again, this method is efficient only for very small molecules, because rotational and translational movements within the active site invariably lead to collisions with the protein and grossly distorted geometries. An application of this strategy can be seen in the work of Goodsel and Olson[11] who used torsional rotations together with rigid-body rotations/translations to dock flexible molecules into rigid protein binding sites. Another approach to increase the efficiency of the search is to gradually reduce the temperature from an extremely high initial value (k_bT = 100 kcal/mole) to a final value near room temperature. This 'simulated annealing' like protocol[12] allows the ligand to explore a range of binding modes before 'annealing' into a particular low-energy mode. The procedure of 'simulated annealing' generates a single refined structure. This can be remedied by repeating the simulations many times or by using the procedure of Hart and Read -Multiple Start MC (*MSMC*)[13] – where a large number of randomly generated starting positions may be used.

Monte Carlo Minimization (MCM)

Replacement of motion in Cartesian space by torsional coordinate movements helps to preserve bond lengths/angles (minimizing distortions in the geometry), but this still leads to collisions with protein atoms, particularly with a tightly packed protein binding site. Li and Scheraga introduced *MCM* that refines the perturbed structure by minimization before applying the Metropolis criteria[14]. The minimization disrupts the Boltzmann distribution, however in docking studies the goal is not to achieve a thermodynamic *ensemble* but to search the relevant conformational space rapidly. One problem with Metropolis MC is that it produces only a single structure. The *MCMM* procedure of Still *et. al.* retains a number of structures through the calculation, any of which can be selected for random variation and energy minimization[15]. Various selection strategies such as energy based, which searches some low-energy regions while neglecting others, or least-used low-energy structures, help give a more uniform coverage of the configuration space. The *MCMM* strategy has been incorporated into the *MacroModel BATCHMIN* program. In Brownian MC (*ICM* of Abagyan *et. al.*)[16] random rotations are scaled according to the radius of gyration or the moment of inertia tensor, so

that molecular rotations and translations give similar displacements to atoms of similar type.

Docking with Genetic Algorithms (GA)

GA is based on ideas from genetics and natural selection, in which a population of individuals evolves over many generations under some selective procedure[17]. In a GA simulation, each individual is associated with a single 'chromosome' represented by integers generally 'ones' and 'zeros'. A fitness function translates the chromosomes into a number effectively evaluating the 'fitness' of the individual. In each generation, the less-fit individuals are eliminated from the population. Pairs of surviving individuals are 'mated' leading to children with chromosomes derived from the parent by mutation or recombination. In point mutations, a randomly selected integer in the chromosome is incremented by a random amount. In the recombination or "cross-over" events, randomly selected homologous segments of the parent chromosomes are interchanged. Off-springs may be obtained by one or more point mutations or recombination. The best individual(s) as assessed by the fitness functions may be passed unchanged to the next generation. The above process may be iterated over many generations, leading to a population with increasingly fit individuals.

In docking applications[18,19] the chromosome encodes the position, orientation and conformation of the ligand. The conformation is represented by a list of torsional angles, stored as an integer or "gray-coded" binary format. The position and orientation are typically represented by a translation vector and Euler angles. The position and orientation may also be represented by superimposing ligand atoms on target points in the enzyme (Oshiro and co-workers)[19]. The ideal fitness function is the total energy of the ligand-protein system, and many variations on this theme abound. Mutations are effected by randomly perturbing the x, y and z translations, the three Euler angles and the torsional angles. Recombination events transfer co-ordinates between chromosomes. This is generally the set of co-ordinates corresponding to a low-energy sub-structure from one copy of the ligand to another. The mutation rate is generally much lower than the recombination rate.

From the above discussion it is apparent that there are many similarities between GA and MC. The probabilistic selection of individuals according to the fitness function in GA corresponds to acceptance or rejection under the Metropolis criterion in MC. Mutations in GA correspond exactly to the random variation operation in conventional MC. However, recombination has no counterpart in MC and is unique to the GA implementation. The explicit use of a population of individuals with different orientations and conformations is also followed in *MCSS*.

Docking with Distance Geometry (DG)

DG is a method of converting interatomic distances into 3D coordinates. The full theory and implementation of DG in chemical problems may be found in reference 20. Blaney and Dixon illustrate the use of DG by docking an ester substrate into the active site of chymotrypsin with use of specific distance constraints between the protein and ligand[21]. The protein was represented by 55 atoms from the active site plus a single dummy atom near the center of a hydrophobic pocket. The upper and lower bounds for these atoms were set

equal. The upper and lower bounds for the substrate were determined from the covalent structure, allowing all rotatable bonds to vary. To prevent interpenetration of ligand atoms into the protein, the lower bounds for the protein substrate distance were set equal to sum of van der Waals radii. Then, a phenyl ring in the substrate was constrained to lie within 2 Å of the dummy atom in the hydrophobic pocket. The ester group was placed near the catalytic residues Ser195 and His57 and the oxyanion hole. This translated into 5 additional distance constraints. Application of DG to this distance matrix lead to several very similar bound structures for the ligand.

Systematic Search Algorithms (SCS)

All the foregoing methods do an irregular search of the conformational and orientational space. None of them guarantee that the global minimum has been located. One can search the space comprehensively by running the methods to saturation, but it is clearly inefficient to discover the same structure over and over again. Docking using SCS requires besides sampling all rotameric states, additional searches over ranges of x, y and z coordinates and over the full range of rotations corresponding to the three Euler angles. A good example of this method is the work of Pincus and co-workers who have docked a flexible disaccharide into the active site of lysozyme[22]. The axis was defined along the length of the active site cleft. Translations along the z axis and rotations about the z axis were described with step sizes of 3 Å and 30° respectively. Because the cleft is quite narrow, the x and y translations were limited and corresponding (x, y) rotations confined to a 45° cone. Grid searches for the six co-ordinates were carried out for each of the three low-energy conformations of the disaccharide molecule. There were 13 structures with energies within 5 kcal of the best structure and were refined by energy minimization. The best structures occupied the sites expected experimentally.

Docking Peptides with the Buildup Approach

This is best described by the *GROW* algorithm[23]. First an amino acid library was generated by running 5,000 cycles of MC minimization on each of the 20 standard amino acids represented as N-acetyl N'-methylamides. The minimization was not taken to completion, leaving slightly distorted structures around each local minimum. Redundant conformations were eliminated leaving for *e.g.* 53 structures for Pro, 171 for Ala, 4,987 for Arg. The construction of the peptide begins from an anchoring amide bond unit pre-positioned in the binding site. The *GROW* procedure attaches the first amino acid to this anchor unit, by superimposing its N-terminal amide onto the corresponding atoms of the anchor amide. Alternately, growth can also proceed in the opposite direction by superimposing the C-terminal amide. The *GROW* procedure iterates over a large number of randomly selected amino acid conformations in the library, each time estimating the interaction with the protein, and saving the 10 to 100 conformations that give the best energy which is evaluated by the *AMBER* forcefield incorporating a solvation model. The procedure is then repeated for the second residue, third and further residues until the peptide assembly is complete. The lack of minimization in the *GROW* program does make it very sensitive to the initial position and orientation of the anchor unit. In its

'unrestricted' mode, *GROW* iterates over alternative amino acids at each stage of the buildup, thus serving as a *de novo* design program, searching for the best amino acid sequence and conformation.

Docking General Organic Molecules with the Buildup Approach

The *GROW* procedure has been extended to use several non-amide linkers to combine molecular fragments, which are selected from the Cambridge Crystallographic Database with specific linkers. Another program is *LUDI* which in one mode of operation[24,25], can position library fragments to make bonds to specified positions on an anchor fragment, without restriction to amides or other special bond types. This is an advantage because it permits construction of a wider range of molecules. However, the disadvantage is that some of the molecules may be difficult to synthesize. The program iterates through the fragment library, tentatively adding each fragment successively to each allowed binding position on the anchor and then estimating the binding energy using an empirical scoring function. In its present library, fragments number about 1,100, which exist as a single conformation or as a very small number of conformations. LUDI is also suited as a *de novo* ligand design.

Whereas *GROW* and *LUDI* assemble molecules from relatively large fragments, *GroupBuild, Sprout, Chemical Genesis* use small fragments and *Legend* and *GrowMol* build molecules from individual atoms.

Having elaborated on the principles behind the various docking methodologies, we now describe our work on the use of docking methods to predict the binding characteristics of the few known inhibitors of HIV-1 Tat protein. It is interesting to note than many of the techniques discussed above have been adopted in our docking studies

Application to HIV-1 Tat Protein

The Tat protein encoded by the human immunodeficiency virus type 1 (HIV-1) is a potent transactivator of gene expression from the viral long terminal repeat (LTR)[26,27]. Tat is a small protein of 86 to 102 residues, with a variable C-terminal size, varying with the HIV-1 isolate. The structure of Tat protein has been divided into several functional domains. The domains that are essential for transactivation are a Pro-X_{AA3}-Pro triad, a cysteine-rich metal binding sequence, and a cluster of basic residues, present within the 57 residue N-terminal of the protein. The first inhibitor of Tat-mediated transactivation, possessing anti-HIV activity, was reported to be *D*-penicillamine (*D*-Pen), a structural analog of cysteine[28]. It was hypothesised that *D*-penicillamine interacts with the cysteine rich region of HIV-1 Tat protein by forming inter-disulfide bonds, thus blocking the transactivation process, which in turn inhibits expression of other structural genes. Other compounds, which interact *via* a similar mechanism, are 2,3-dimercapto-1-propanol (DMP) and N-(2-mercaptopropionyl)-glycine (MPG)[29]. The transactivation of LTR (HIV-1) by the Tat protein was studied in Jurkat cells in the presence of penicillamine, DMP and MPG individually. It was found that *D*-penicillamine is the most potent inhibitor of HIV-1 Tat. DMP shows anti-HIV-1 Tat activity but it is toxic at the dose level where activity persists. The anti-HIV-1 activity of MPG is seen in high concentrations; however, it does not exhibit any toxicity

even at these high concentrations. For the last two mentioned inhibitors, there is no information on binding modes and the relationship between stereochemistry and biological activity as exists for penicillamine. In view of this, we have attempted to predict the relative affinities of the stereoisomers of both DMP and MPG and also try to rationalize the vast differences in the level of activities and toxicities of penicillamine, DMP and MPG by docking methodologies using *DOCK* (v 4.0)[3] and *Affinity* (Accelrys, USA) programs.

Docking methodology adopted for HIV-1 Tat protein

The structure of HIV-1 (Zaire-2 isolate) Tat protein determined by NMR and deposited in the Protein Data Bank was used for our docking studies[30]. The system was prepared to work at physiological pH with its total charge as zero. The protein structure was minimized using the CVFF forcefield[31] with the protein backbone atoms tethered to their original positions by a force constant of 100 kcal/mol/Å^2. The gradient tolerance for minimization was set to 0.01 kcal/mol/Å.

In HIV-1 Tat protein, the cysteine rich region covering residues 20 to 38 has been suggested as the putative binding site for penicillamine. The molecular surface (Connolly Surface) covering these residues was prepared by the *SYBYL* program *MOLCAD* (Tripos Inc., USA), with a probe radius of 1.4 Å. The program *SPHEGEN* (DOCK v 4.0) was then used to construct shape based site points and with their associated normals to determine spheres that fill the site. These spheres were then grouped into clusters. After refinement, a cluster of 46 spheres (the 'target points') was enclosed in a grid of dimensions 34 x 34 x 29 Å^3 with a 0.3 Å spacing. On this grid is stored the steric and electrostatic information of the receptor atoms which is used for 'force field scoring' of the orientations determined by *DOCK*. The program *DOCK* was used to identify binding sites and binding orientations by superimposing four randomly selected atoms of the ligand onto the 'target points' describing the binding pocket of the enzyme. The various binding modes so determined by *DOCK* can be scored based on shape complementarity or energy which is calculated as the sum of van der Waals (Lennard Jones) and electrostatic (Coulomb) energies. We have used energy criteria to rank the various binding modes for the two isomers of penicillamine, DMP and MPG.

The '*DOCK* best scored structures' for each isomer of the three inhibitors, were then subjected to more accurate binding studies by *Affinity*[32]. It is an energy driven method that uses a combination of Monte Carlo (MC) and Simulated Annealing (SA) techniques to determine binding modes. Random displacements of the ligand within the binding site are made by a MC procedure, followed by SA that helps to cross energy barriers, thus optimizing the docking of ligands in the active site. The binding site was defined as a 5 Å region from the centroid of the *DOCK* generated structure of *D*- and *L*-penicillamine. Docking by *Affinity* was initiated with the Monte Carlo minimization approach. During this phase, the structures were moved by a random combination of translation, rotation and torsion changes. The maximum permissible translational movement of the ligand was set to 1.0 Å, and the maximum angle of rotation was placed at 180°. The random moves sample both the orientation and conformation space of the ligand with respect to the receptor.

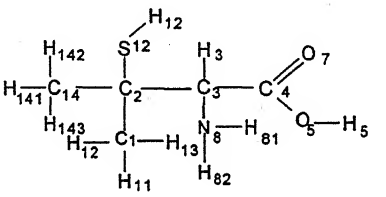
Random placement of the ligand in the binding pocket can potentially lead to severe strain in the Coulombic and van der Waals (vdw) energies and rejection of many configurations. Thus during this stage, a simple quartic form for the Lennard-Jones potential was used, with the Coulombic energy neglected. At the end of the move, if the energy of the ligand was within 200 kcal/mole of the lowest energy structure, it was retained, otherwise rejected. About 100 such MC moves were made, and each time, the energy of the ligand was evaluated, and the structure accepted or rejected according to the criterion mentioned above. The resulting pool of structures from the MC process was then energy minimized. At this stage the structures were screened using a standard Lennard-Jones potential and the more accurate Cell Multipole method for the electrostatics[33]. The ten 'best structures' were then further refined by simulated annealing which terminated with a final round of minimization of the annealed structures. During simulated annealing the temperature was decreased linearly from 500 K to 300 K in steps of 4 K over a period of 5000 fs. The structures were then energy minimized by 1000 steps of conjugate gradients to a gradient tolerance of 0.001 kcal/mol/Å.

Since penicillamine, DMP and MPG, are flexible molecules, with quite a few torsional degrees of freedom, we have used Systematic Search (*Insight II*, Accelrys Inc. USA) to map the conformational space of the two isomers of each molecule. A grid search was done, where torsions were varied in 30° increments and the generated structures energy minimized. Finally only unique structures were kept (structures were compared based on rmsd of heavy atoms), which were then docked into the Tat active site with the *DOCK* program by the protocol described above.

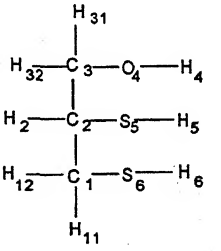
Results and Discussion

Systematic Search for both isomers of penicillamine, MPG and DMP identified some unique conformations. The conformation with the lowest energy was then selected and used for a 'more refined' *DOCK* (flexible docking) procedure, where during this docking process, variation in the torsional angles was also considered. From the various possible binding modes discovered by flexible docking, the best *DOCK* structures for the two isomers were separated and used as starting points in the *Affinity* program.

Penicillamine



2,3-Dimercapto-1-Propanol (DMP)



N-(2-Mercaptoproponyl)-Glycine (MPG)

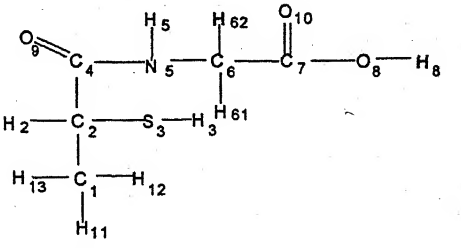


Fig. 1– Atom labels of Penicillamine, DMP and MPG.

The docking study revealed that all possible binding orientations for *D*- and *L*-penicillamine clustered in two regions in the Tat active site, which we have labeled as **A** and **B** (Fig. 2 and 3). In case of *D*- and *L*- DMP a third binding region identified as **D** exists, while for *D*- and *L*-MPG still another region marked **C** seems favourable for binding. The amino acid residues composing regions **A, B, C** and **D**, within a 6 Å radius from the centre of mass of the inhibitor are listed in Table 1. The binding energies calculated by *DOCK* for the two isomers of penicillamine, DMP and MPG (Table 2) are nearly the same, showing that *DOCK* is unable to distinguish the binding of the two isomers to Tat. These results are not surprising, as *DOCK* does not use a very accurate force field for the energy estimation. It is for this reason that the '*DOCK* best scored structures' of both isomers of the three inhibitors of Tat were subjected to more accurate binding studies by *Affinity*. The results of *Affinity* studies are summed up in Table 3. It is evident from this table, that the true binding energies of the *D*- and *L*-isomers in all regions are clearly differentiated, with the *D*-isomer predicted to bind more firmly than the *L*-form. It can also be seen that, for both isomers of pencillamine, site **A** is the preferred region, with the *D*-isomer favoured over the *L*-form. For all molecules, the **B** region seems the least favoured. In case of DMP, the **A** and **D** regions are equally preferred for binding. Concerning MPG, the *L*-isomer has a slightly higher affinity in the **A** region, but the *D*-isomer scores over the *L*- in the **D** region. In summation, for all three inhibitors, the *D*-isomer has a higher affinity that the *L*-form. A break-up of the components of the binding energy for the *D*- and *L*-isomers shows that it is the van der Waals interaction, which accounts mainly for the differences in their binding energies. Our predictions for the isomers of pencillamine are in accordance with experimental evidence[34].

The hydrogen bonds made by the two isomers of each inhibitor with residues in the active site of Tat are mentioned in Table 4. It can be seen in this table that H-bonding with Cys^{22} is prominent in region **A**, with Cys^{31} and Cys^{34} in region **C** and with Cys^{27} in region **D**. It is also remarkable that in region **B** only the *D*- isomer (and not *L*-) of both penicillamine and MPG shows H-bonding with Cys^{22} while H-bonds with Cys^{22} occur for both isomers of DMP. Thus supports the mechanism of action of penicillamine which is postulated to result from formation of covalent linkages with cysteine residues in the various regions. This mechanism of action can be safely extrapolated for the other two inhibitors – DMP and MPG.

Table 1– Composition of Sites **A, B, C** and **D** deemed favourable for binding the HIV-1 Tat inhibitors

Site A	Site B	Site C	Site D
Trp^{11}, Asn^{12}, Ala^{21}, Cys^{22}, Asn^{23}, Arg^{24}	Pro^{10}, Trp^{11}, Cys^{22}, Asn^{23}, Arg^{24}, Gln^{35}, Val^{36}	Cys^{31}, Tyr^{32}, His^{33}, Cys^{34}, Gln^{35}, Gly^{61}, Gly^{62}, Gln^{63}	Cys^{25}, His^{26}, Cys^{27}, Lys^{28}, Cys^{34}, Gln^{35}, Thr^{40}

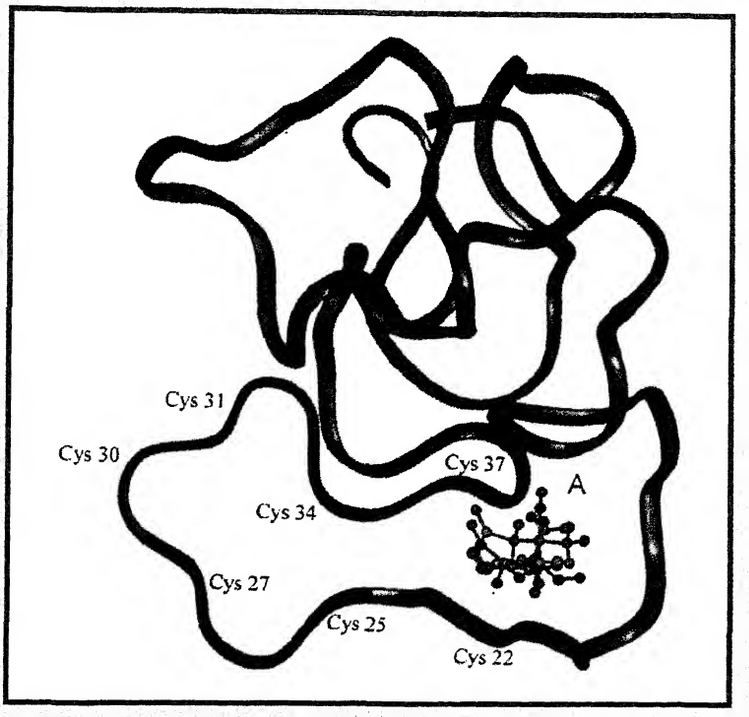


Fig. 2 Binding orientation of *D*-penicillamine (green) and *L*-penicillamine (red) in region A of HIV-1 Tat protein. The protein backbone has been drawn as a ribbon and the cysteine residues in the protein are also indicated.

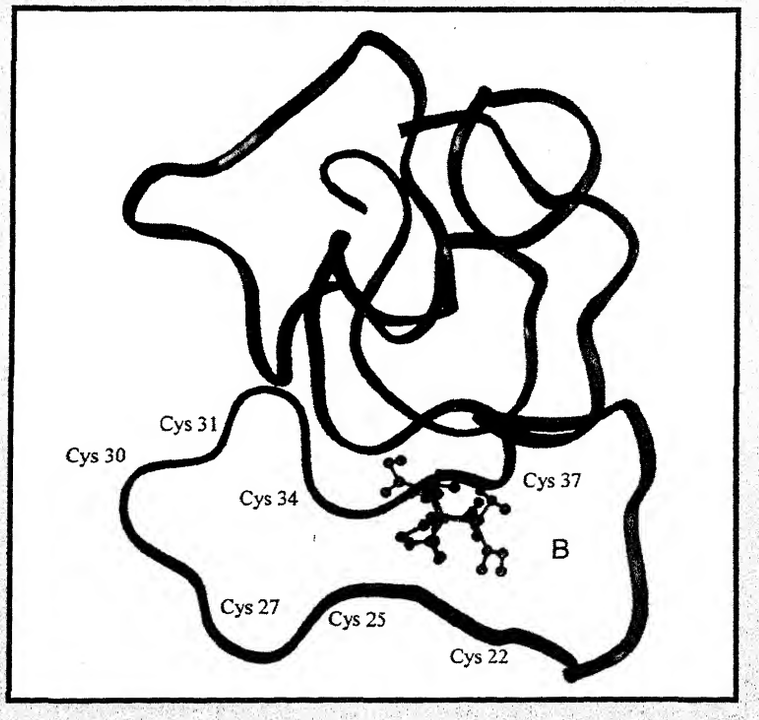


Fig. 3. Binding orientation of *D*-penicillamine (green) and *L*-penicillamine (red) in region B of HIV-1 Tat protein. The ribbon designates the protein backbone with cysteine residues also indicated.

Table 2– Binding energies of *D*- and *L*-isomers of penicillamine, DMP and MPG by *DOCK*

Region	Energy (kcal/mole)					
	Penicillamine		DMP		MPG	
	D	*L*	*D*	*L*	*D*	*L*
A	-14.4	-14.6	-11.4	-11.1	-16.6	-15.5
B	-13.8	-13.8	-10.7	-9.9	-15.8	-14.4
C	-	-	-	-	-15.1	-16.5
D	-	-	-10.2	-10.3	-15.4	-15.1

Table 3– Binding energies of D- and L-isomers of penicillamine, DMP and MPG by Affinity

Region	Energy (kcal/mole)					
	Penicillamine		DMP		MPG	
	D	L	D	L	D	L
A	-11.5	-7.8	-8.4	-7.2	-13.1	-13.6
B	0.1	9.8	3.8	5.3	-8.1	-6.9
C	-	-	-	-	-13.1	-4.6
D	-	-	-8.9	-7.5	-15.6	-13.8

Table 4– H-bonds between the inhibitor† and the protein HIV-1 Tat

Inhibitor	Site A	Site B	Site C	Site D
D-Pen	N^{8}...H-N(Cys^{22}), H^{5}...O=C(Cys^{22}),	N^{8}...^{5}H-N(Arg^{24}), H^{5}...O=C(Cys^{22})	-	-
L- Pen	N^{8}...H-N(Cys^{22}), H^{5}...O=C(Cys^{22}),	N^{8}...H-N(Val^{36}), H^{82}...^{5}H-N(Arg^{24}), O^{7}...^{e}H-N(Gln^{35})	-	-
D-DMP	H^{4}...O=C (Cys^{22})	H^{4}...N-H(Val^{36}), O^{4}...N-H(Val^{36})	-	H^{4}...O=C(Cys^{27})
L-DMP	H^{4}...O=C (Cys^{22})	H^{4}...N-H(Val^{36}), O^{4}...N-H(Val^{36}), S^{5}...^{e}H-N(Gln^{35})	-	H^{4}...O=C(Arg^{24})
D-MPG	O^{9}...H-N(Cys^{22}), H^{8}...O=C (Cys^{22})	S^{3}...N-H(Val^{36}), O^{9}... ^{5}H-N(Arg^{24}), O^{10}...H-N(Cys^{22})	H^{8}...O=C (Tyr^{32}), O^{8}...H-N (Tyr^{32}), O^{9}...H-N (Lys^{29})	H^{5}...O=C (Cys^{34}) O^{9}...H-N (His^{26}), H^{8}...O=C (Arg^{24})
L-MPG	O^{9}...H-N(Cys^{22}), H^{8}...O=C (Cys^{22})	H^{8}...O=C (Cys^{22})	S^{3}...H-N (Gln^{35}), S^{3}...H-N (Cys^{34}), H^{8}...O=C (Cys^{31})	H^{5}...O=C (Cys^{34}) O^{9}...H-N (His^{26}), H^{8}...O=C (Arg^{24})

†The atoms of Penicillamine (Pen), DMP, MPG have been labelled in Fig. 1.

Conclusions

Docking studies have helped to identify the putative binding modes for both the *D*- and *L*- forms of penicillamine, MPG and DMP. It also rationalizes the differences in activities of the two isomers in terms of their binding orientations and energies. The study also corroborates the experimental finding that *D*-penicillamine is a more potent inhibitor of tat-mediated transactivation than the *L*-isomer[10]. This work also supports the mechanism of action of these inhibitors as resulting from possible covalent linkages with cysteine residues in HIV-1 Tat protein. As mentioned earlier, pencillamine is the most potent inhibitor of HIV-1 Tat protein. DMP is toxic at doses where it shows activity, while MPG is active at very high doses but non-toxic at these levels. These differences in the activities may be associated with their binding affinities and preference for binding to selective regions in the Tat enzyme as discussed above. To be specific, the binding of DMP and MPG to additional sites **C** and **D** which may be less efficient in transducing the binding signal into a response, may account for both their high dose requirements and/or their toxicities. Thus docking studies offer an interesting approach to understand how structural modifications in ligands can influence their ability to block signal functions of the Tat protein of HIV-1, leading to ideas for optimizing their binding affinities.

Acknowledgments

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Mapping from high resolution satellite images and global positioning systems

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Abstract

The development in space based imaging and the sophisticated image processing techniques, during the past three decades, have revolutionized the field of cartography and mapping. The present paper describes the recent advances in the Satellite Remote Sensing, Global Positioning System and GIS as well as their uses in the process of mapping. Studies on preparation of thematic as well as cartographic quality base maps using satellite images and GPS have been described.

(Keywords : mapping/satellite remote sensing/ global positioning system/ thematic mapping/ cartography).

Preparation of development plan often requires a variety of spatial thematic information (maps) on desired scale as well as a number of attribute / non spatial information. The thematic information comprises of spatial data on natural resources, topography and infrastructure / communication network whereas the non spatial information, mainly comprise of the demographic patterns and socio economic profiles of the area. Development plan, in both rural as well as urban sector, requires creation and integration of above mentioned spatial and non spatial data layers in GIS (Geographic Information System) environment using criteria based decision rules. This is normally done by georeferencing the set of the thematic layers on different natural resources using a common base map. The base information are normally extracted from the available topographical maps.

Plans are normally prepared at different hierarchical levels i.e. regional, district / taluka or village level, depending upon the need. Requirement of scale and the information contents depends on the levels of planning. For example, the mapping scale required at state level is 1:250,000 – 5,00,000; at district level it is 1:50,000, and at local/village level it is 5000-15,000 scale. Preparation of development plans at different levels, thus, requires generation of i) thematic maps and, ii) base maps/topographic maps on the desired scale. Both these categories of maps are two dimensional representation of the parts of the earth's surface containing basic physical and/or cultural features. The topographical maps depict the physical characteristics and features likes roads, rail, settlements, drainage, water bodies, broad land use/land cover, administrative boundaries etc. and the information on surface reliefs represented by contour and spot heights. Topographic maps are used as a base in developmental activities and hence it is essential that they are reliable and

accurate. These maps, in our country, are generated by Survey of India on 1:25,000, 50,000 and 2,50,000 scales with contour intervals of 10, 20 and 100 meters, respectively. Sometimes, need specific large scale topographical maps (1:10,000 scales) are also prepared.

Thematic maps, in the present day context, refer to the maps depicting the details on a particular type of information, such as land use/land cover, soil, ground water potential, drainage, transport network etc. The scale, information content etc. of the thematic maps depends, largely on the purpose for which they have been prepared. These maps are required for area development planning at various hierarchical levels i.e. regional, district, village and implementation level. As an example, the thematic maps on land use / land cover, soil, hydrogeomorphology (ground water prospect), drainage and surface water bodies, slope, transport network, settlement and watershed boundaries are used in conjunction with the socio-economic profiles of the area for preparation of land and water resources development plan in the context of rural development. Planning at district level would require thematic maps on 1:50,000 scale. Micro-level planning i.e. the planning at implementation level requires thematic and base information at a larger scale. The scale of the required spatial information for micro level planning, varies from, one is to few hundred to a few thousands depending upon the needs and purpose of plan preparation. For example, in rural sector, the largest scale base maps i.e. the cadastral maps are available on 1:4000 / 8000 / 16000 scale. These maps are used for implementation work by planners and implementing agencies. In urban sector, the zonal planning and town planning schemes are prepared on 1:500-5000 scale depending upon the size of the planning area and also the specific needs.

The most important element in the process of map making (both topographical and thematic) is data capturing. The accuracy and reliability of a map depends upon the quality of data used. Thus, the data capturing techniques needs to be reliable, efficient and cost effective. The technique adopted should be able to meet the requirements of information content and accuracy of the maps to be prepared. Traditionally, ground / field surveying has been the most common method of data collection and has been in practice since the initial days of map making. It is very well known that the ground based surveying takes a lot of time and efforts, and is costly too. The aerial photographs, as a data capturing / acquisition system, has been in use for the last five decades. It has been extremely useful, time efficient and cost effective as compared to the tedious ground surveying for data collection. In fact, the aerial survey has augmented the pace of mapping with the use of sophisticated photogrammetric techniques during the last few decades.

Recent advances in the space based data capturing techniques (imaging), during the past three decades, have revolutionized the field of cartography and mapping. High resolution images acquired by satellites, global positioning systems (GPS) as well as the sophisticated image processing and GIS techniques, developed during the last decade, have further augmented the pace of mapping and its updation manyfolds. The use of satellite images for mapping started way back in 1972 with the launch of ERTS

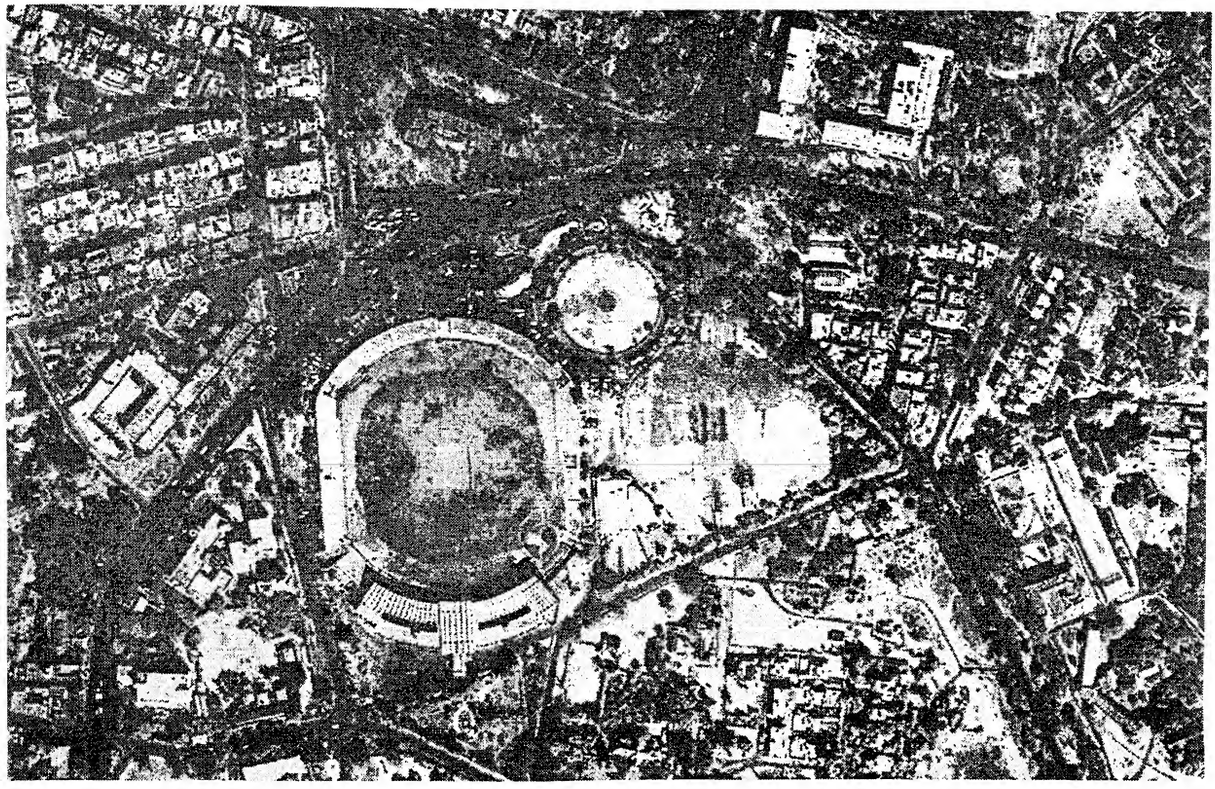

Fig 1– 1m PAN and 4m multi spectral merged image from IKONOS satellite for part of Indore town showing stadium, roads and vehicles on it, buildings, trees, gardens etc.

satellite (later known as LANDSAT), the first one in the series of earth resources satellites. The period of past three decades has witnessed a dramatic improvement in spatial resolution from 80m to less than a meter, monoscopic data capturing to stereoscopic imaging, broad band to hyper spectral bands and medium radiometric resolution to high radiometry. All these improvements in satellite imaging has led to availability of better quality images for mapping applications. Very high resolution image taken from IKONOS satellite for parts of Indore town is given in Fig. 1. It shows the details of urban features such as stadium, buildings, gardens, tree, roads including vehicles on it. It is a false colour picture, known as FCC, where green vegetation is depicted in red colour, buildings and roads in bluish grey colour. There have been rapid strides in the imaging / data capturing capability from space platform in our country as well. The improvement in indigenous space imaging quality, in terms of spatial resolutions from 1 km in 1979 (offered by Bhaskara) to as high as 1m in 2001 (TES), as well as the stereoscopic capabilities from IRS 1C and 1D have given tremendous boost to the field of mapping in the country. The Cartosat I (scheduled for launch in October 2004) and II programme of ISRO, with 2.5 m and 1m spatial resolution along with the stereo capability will go a long way in empowering and strengthening the discipline of mapping and surveying in the country by offering mapping potential to a scale of 10,000 scale for topographic and to 4, 000 scale for thematic mapping.

Topographic Mapping :

Topographic mapping from space data began with the launch of French satellite,

SPOT 1 in 1986. One of the important requirements of topographic mapping (in addition to feature extraction) is the information on height and relief, represented by contours in the map. The object / feature identification capability of the satellite images depend upon its spatial resolution (horizontal resolution), imaging condition and the scene contrast, where as the contour intervals to be derived from the stereo images depend upon its height accuracy (vertical resolution). The height accuracy depends, mainly, on the horizontal resolution and the base to height ratio (B/H). The relationship is given by

$$\text{Height accuracy} = \frac{\text{Horizontal resolution}}{\text{B/H}}$$

For planimetric representation, in hilly and undulating terrain, the height information is required for orthorectification of the images. Orthorectification is the process of removing all kinds of geometric distortions from the image, including the relief distortion. Removal of relief distortion requires height information on pixel by pixel basis i.e. the DEM of the areas[1]. DEM can be generated either by photogrammetric techniques using stereo image pairs or through interferometric technique using Synthetic Aparter Radar (SAR) data. Thus, for the planemetric representation, stereo capability is not required if the height information (DEM) is available from other sources. An orthophoto can be used for monoplotting, but nevertheless a stereoscopic view support the better object / feature identification.

Object / feature identification from the image depends, not only on the pixel size (spatial resolution) but also on the spectral information. Thus with the same spatial resolution, the multispectral images will have better object / feature identification than the panchromatic images. Object identification also depends on the scene contrast, imaging conditions and radiometry.

In the context of topographic / cartographic quality mapping, as a thumb rule, the pixel size on the ground shall not exceed 0.05 to 0.1mm in the map scale[2]. The range between the lower and upper value of this rule depends upon the structure / heterogeneity of the area, the imaging conditions and the mapping standards to be met. The spatial (horizontal and vertical) resolution requirement of the satellite images for topographic mapping at different scales are given in Table 1.

Table 1– Scale vs. spatial and height resolution requirement for topographic mapping

Mapping scale	Spatial resolution (pixel size)	Height resolution (contour Intervel)
1:50,000	2.5 - 5.0 m	< 8m, (20m)
1:25,000	1.25 – 2.5 m	< 4m, (10m)
1:10,000	0.5m – 1.0 m	< 1.5, (4m)
1:4,000	0.2 m – 0.4 m	< 0.4, (1m)

Cartographic Quality Base map :

A variety of thematic maps such as land use/land cover, soils, hydrogeomorphology /ground water prospect, vegetation / forest, transport / communication network etc. are often required for preparation of development plans. One of the critical requirement, while preparing these thematic maps from satellite data, is a base map. These base maps are primarily required for georeferencing the satellite data and also used as a control for interpretation of various

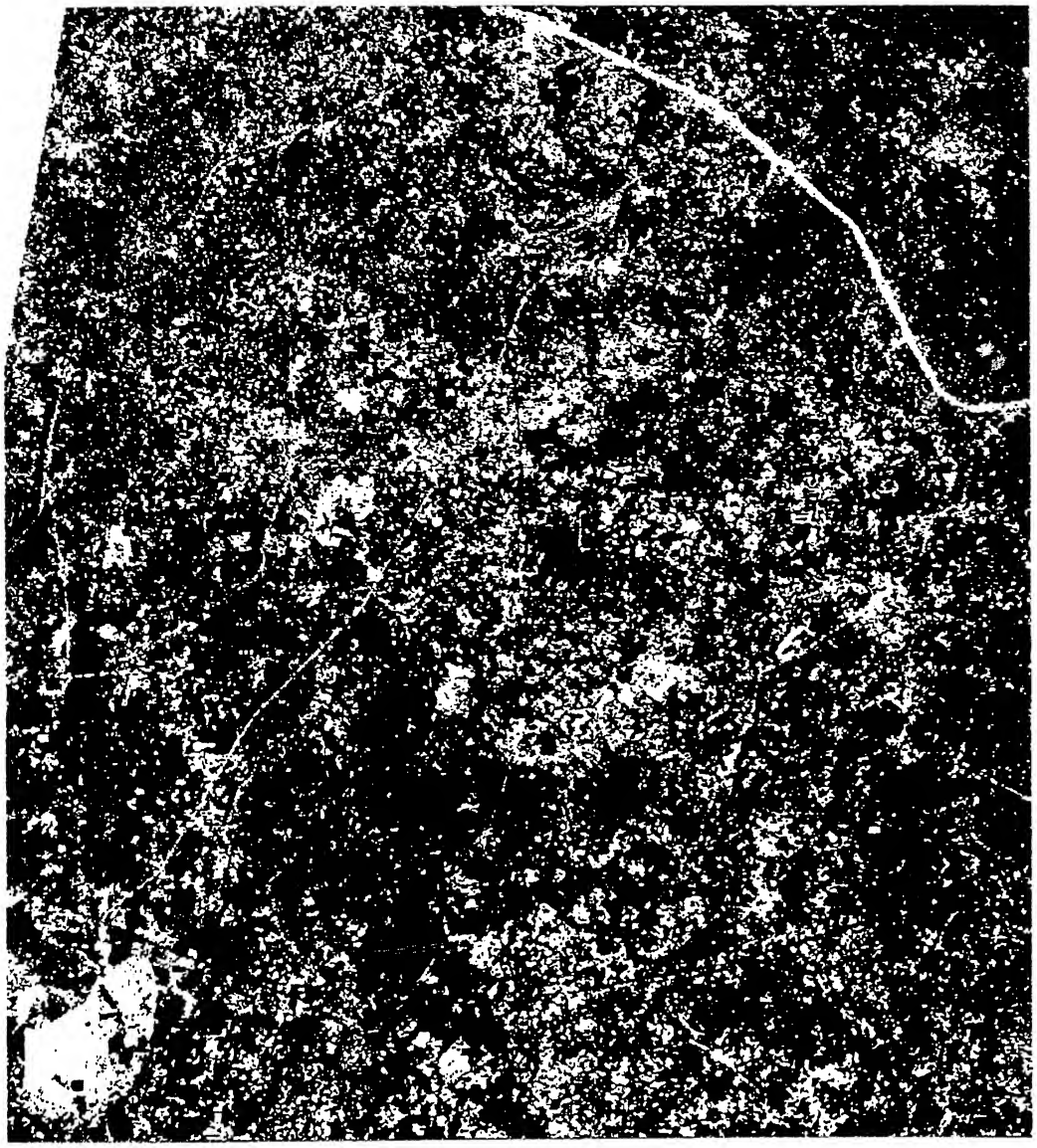

Fig. 2– Geo referenced LISS III + PAN merged image for parts of Ahmedabad and Mehsana (Jan. 23, 2001)

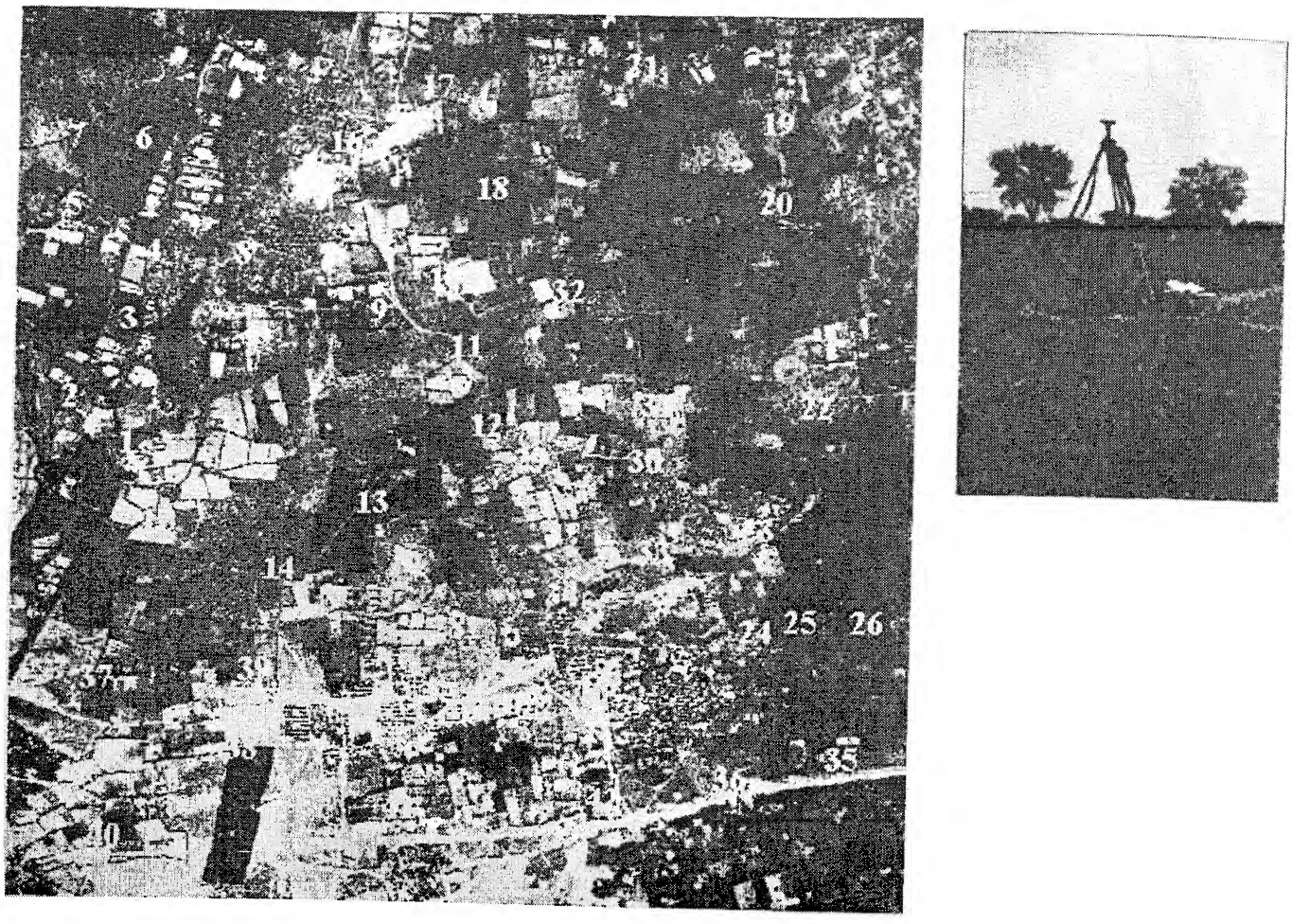

Fig. 4– Satellite image showing GPS locations and GPS receiver at one of rover station .

features *vis-à-vis* its geographical locations. The base maps are georeferenced maps following a standard coordinate system, appropriate projection system as well as the standards in terms of locational accuracy, tolerance etc. The base maps, normally contain a few major features like roads, railway line, settlement/towns, water bodies, streams and drainages. In addition, it also contains attribute information on the names of towns, rivers as well as other annotations and co-ordinates.

Cartographic quality base maps with desired accuracy standards can be prepared by interpretation of satellite data with spatial resolution commensurate to the scale, and differential GPS measurements. Satellite images (nadir) can be directly used for feature extraction. However, in undulating/hilly area, the orthorectification of the satellite images using DEM with desired accuracy and grid size, becomes essential. Satellite stereo pair images can be successfully used for preparation of base maps in undulating area. GPS measurements in differential mode is required to establish the accurate coordinates of the control points needed for georeferencing the images. Such exercise has been carried out at Space Applications Centre (ISRO), in the plane terrain of Ahmedabad district[3] and undulating terrain of Alwar[4] and in hilly area of Sikkim[5].

Stereo Panchromatic (PAN) data from IRS-1D has been used for preparation of base maps at 1:50,000 scale in WGS – 84 datum and UTM projection. Differential GPS (Global Positioning System) measure-

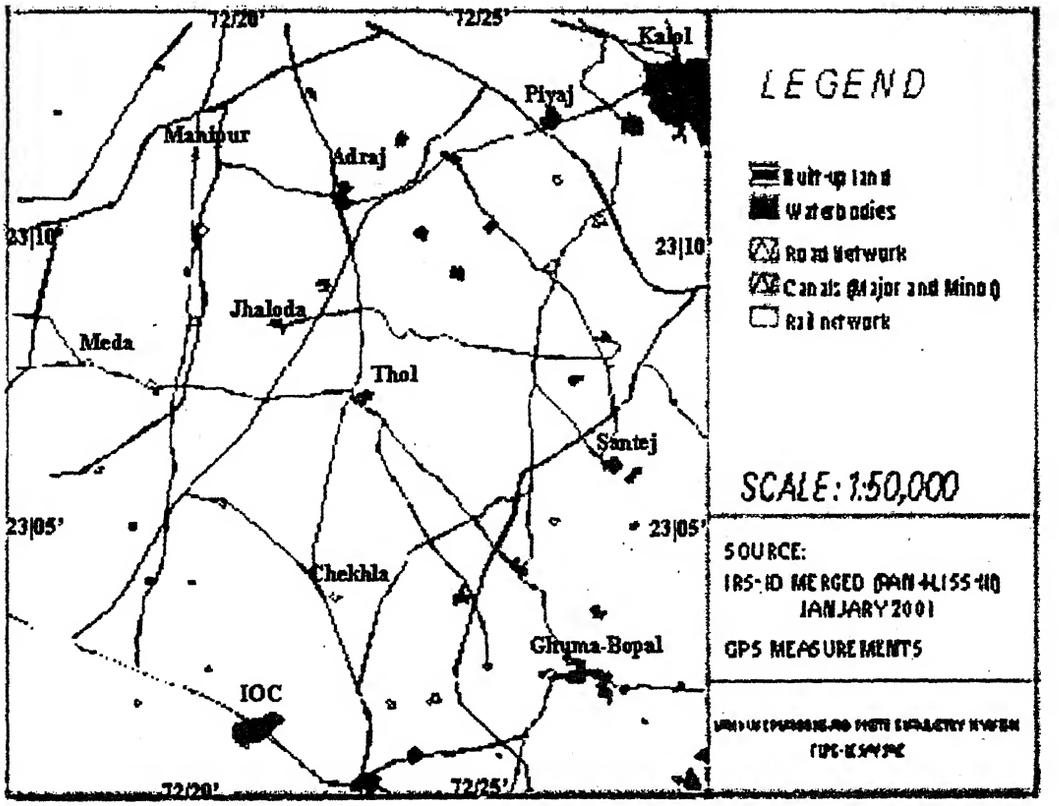

Fig. 3– Base map of parts of Ahmedabad prepared using satellite image and differential GPS measurements.

ments have been used for establishing GCP (Ground Control Point) coordinates, georeferencing the PAN image and accuracy assessment of the maps prepared. IRS 1D LISS III and PAN merged image for parts of Ahmedabad is shown in Fig 2. The base map features, such as major river, road, rail, settlements, water bodies etc. have been extracted through the analysis of IRS-1C image of the study area. The base map (covering 15' x 15' area) prepared in WGS – 84 datum and UTM projection is shown in Fig 3. The cartographic quality of the base map in terms of conformality, equivalence and scale have been evaluated using differential GPS measurements. The RMSE for difference in longitude and latitude between GPS coordinates and that derived from base map, generated from merged product, were 5.45 m and 3.27 m respectively. When LISS-III data alone was used, the RMSE in longitude and latitude difference were 11.5m and 9.8m respectively.

The evaluation of scale variation in the base map indicated the average variation in scale to be 0.062 percent. The comparison of the angles made by baselines with true north as measured on base map and the corresponding base line as obtained with differential GPS measurements indicates average difference of 0.015 percent.

1m spatial resolution data from IKONOS and TES satellites can be used to prepare the cartographic quality maps on the scale ≈ 1:10,000 in a plane area. With the availability of DEM with accuracy better than 2m, the mapping on 1:10,000 scale can be done in undulating area.

A demonstrative exercise on land parcel mapping using 1m data has been attempted at Space Applications Centre for a study site in Ahmedabad[6] The cadastral boundaries extracted from panchromatic data of 1m resolution have been supplemented by the mensruation / plot dimension measurements by total stations and validated with differential GPS measurements. The study on land parcel mapping has been carried out in a flat terrain area of Ahmedabad district covering approximately 16 sq. km. bounded by 72° 32' 0" to 72° 35' 0" N latitude and 22° 55' to 22° 57' E longitude. Differential GPS measurement campaign were mounted to establish the coordinates of ground control points and check points in WGS – 84 datum. Satellite image (1m, PAN) along with the locations of GPS measurement points is given in Fig. 4. Total station instruments have been used to make field observations for the calculation of length and breadth of selected plots. Village maps obtained from state revenue department were used. Satellite data of 1m spatial resolution is georeferenced using the ground control points, in WGS – 84 reference datum, established by differential GPS measurements. The registration accuracy (RMSE) of the image has been found to be 0.2730 m in Easting and 0.4147 m in Northing. Land parcel boundaries, roads, settlement etc. were extracted from the georeferenced image by visual interpretation technique. The land parcel boundaries as extracted from image and those available on the village cadastral map for Lakshmipur village (Ahmedabad) is given in Fig. 5. The number of land parcels and the total area of the parcel from image and that from the village map are 293 nos, 183.45 ha and 155 nos., 181.47 ha respectively.

The comparison of land parcel boundaries as obtained from image interpretation and total station/GPS measurements with the available cadastral map shows (Fig. 5)

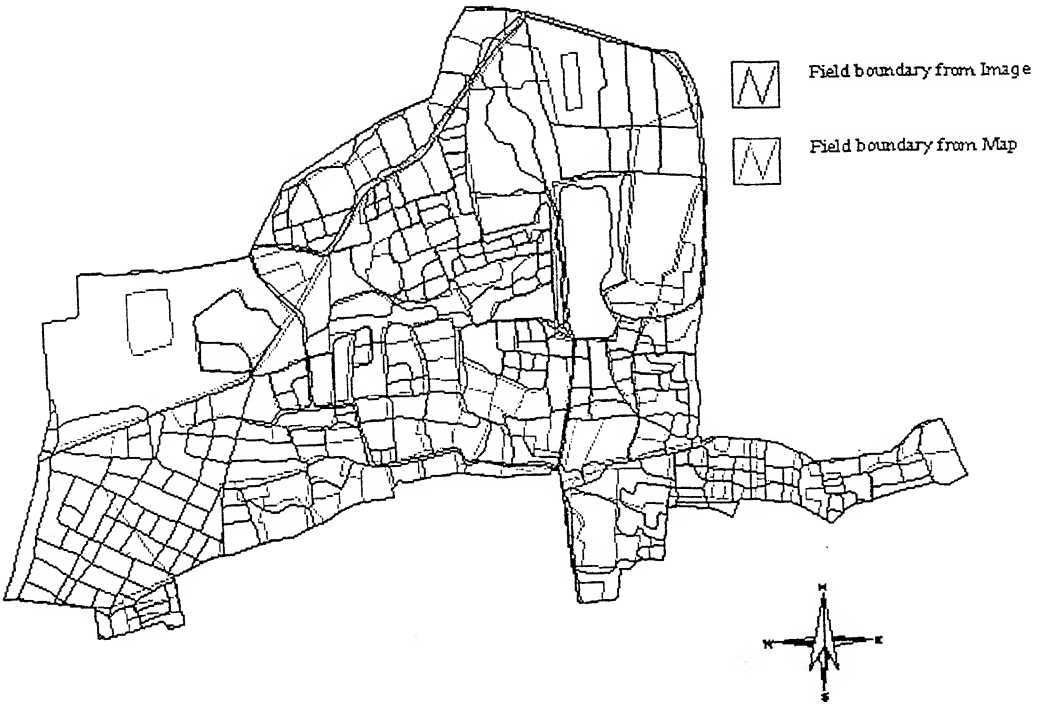

Fig. 5– Field boundaries in Lakshmipur village (Ahmedabad) extracted from geo referenced high resolution satellite image and cadastral map.

that a number of plot boundaries which appear on the map prepared from satellite image do not exist on the cadastral map. This is mainly due to the fact that the cadastral map used is quite old and new sub plot boundaries would have been created (division of plots in two or more) in due course of time. A few plot boundaries are not seen on the image but exist on the map. This is attributed to the fact that some of the land parcel boundaries do not have physical appearance and are only notional boundaries. Such boundaries will not be observed on the images. This exercise clearly brings out the fact that using high resolution satellite images, a tremendous amount of effort can be reduced in surveying for land parcel mapping. The extraction of the plot boundaries as seen on the image supported by the ground surveying using total station and/or GPS measurement could be the best strategy for land parcel mapping. This may save 50 to 60 percent efforts as compared to the mapping totally based on field surveying. However, this will depend upon the terrain conditions, soil type and scene contrast.

Thematic Mapping :

Thematic maps are the important inputs for any kind of area development planning. The examples are watershed development, urban planning, waste land development, combating desertification etc. Requirement for thematic maps, in terms of scale, information contents, classification system/ legends depends on the purpose for which the maps are to be used. Most of the thematic maps required for natural resources management are being prepared, operationally, using satellite data. There

has been sustained improvement in the accuracy as well as the scale of thematic mapping based on satellite data interpretation during the past three decades. It has happened due to continued improvement in the quality of satellite data in terms of spatial, spectral, radiometric and temporal resolutions. Both, visual interpretation and digital analysis techniques are being used for preparation of thematic maps from satellite images.

While preparing thematic maps, the following standards need to be set / evolved.

Scale of mapping

Size of Minimum Mappable Unit (MMU)

Classification System

Legend

The format of the map and its physical size / coverage

The scale of map to be prepared is restricted by the spatial resolution of the satellite data. Larger the scale of the map, higher the spatial resolution required. The dependence of the scale of mapping on the spatial resolution of satellite images is apparent from Fig 6. It shows the urban land uses such as buildings, roads, trees etc. on 1:5,000 scale from 1m, 2.5m and 5.8m spatial resolution images. It is clear that 1m image very clearly and sharply depicts the boundaries of the urban land use classes (see the boundaries of the buildings) at 1:5,000 scale. The boundaries are less sharp in 2.5m image. While the boundaries are diffused in 5.8m images. It indicates that 1m data may be used for preparation of urban land use map on 1:5,000 scale.

Based on our experience, at Space Applications Centre, in preparation of a variety of thematic maps from interpretation of satellite data, the requirement of spatial resolution for thematic mapping at different scales (needed for different level of planning) is given below :

Table 2–Area coverage, scale and spatial resolution for thematic mapping

Area Coverage	Scale	Spatial resolution
Regional	1:1-5 M	200 m – 1 km
National/State	1:250,000	75 – 100 m
District	1:50,000	20 – 40 m
Taluka	1:25,000	10 – 15 m
Village/ implementation level	1:4,000 – 10,000	1 – 5 m

MMU of 3 mm x 3 mm comprises of 10 x 10 pixels

Normally, the Minimum Mappable Unit (MMU) is taken between 2mm x 2mm – 3mm x 3mm at the particular scale. With 3mm x 3mm MMU, the minimum size of the feature polygon which can be depicted in the map on 1:50,000 scale is 2.25 ha. It means any feature having size less than 2.25 ha on the ground will not be shown on the map.

The level of information content represented by classification system is always linked to the scale of map. For example, a land use / land cover map on 1:250,000 scale or smaller, will depict, only level one classes. Whereas the map on 1:50,000 scale will depict the classes upto level II. Thus, a generalization takes place while going from larger scale to smaller scale.

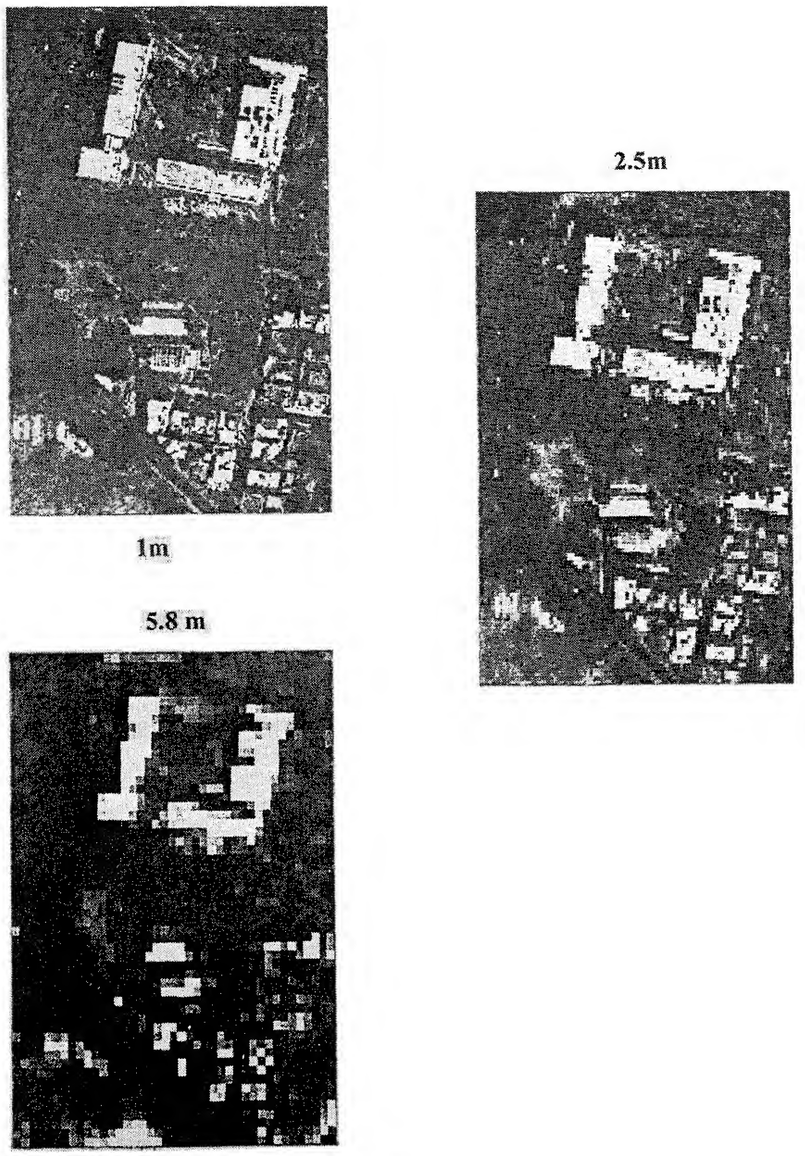

Fig. 6– Satellite Pan images with 1m, 2.5m and 5.8 spatial resolutions on 1:5000 scale showing urban land use classes such as buildings, trees, roads and other features.

A standard existing classification system, commensurate to the scale of mapping, should be used for preparation of thematic maps. National Level Standards on Classification System are available for many themes, such as, land use/land cover, hydrogeomorphology, wetlands, forest type, desertification status, land degradation, soil etc. These classification systems have been evolved through consensus at national level.

Similarly, the standard legend, colour scheme and the format should be used while preparing a particular thematic map. However, the format and physical size (in case of hard copy maps) and coverage may vary depending upon the use and purpose of mapping.

For some of the themes, namely, land use / land cover, wastelands, wetlands, hydrogeomorphology, ground water prospects, forest vegetation (density) and soils, mapping have already been done for the entire country. These have been executed by the concerned national organizations.

Today, with the availability of 5.8m multispectral data from IRS-P6 (Resourcesat) and 1m panchromatic data, the capability of mapping (thematic) exists upto a scale of 1: 5000.

Conclusions

The field of cartography and mapping (both, topographic and thematic) have developed over a period of time. The pace of development have accelerated during the past three decades due to availability of data from earth observation satellites. The technological strides in satellite based imaging in terms of spatial, spectral, radiometric and temporal resolutions has revolutionized and strengthened the mapping process by providing the opportunity of mapping at larger scale with desired accuracy in shortest possible time.

With the available 23 m multi-spectral in combination with 5.8 m panchromatic data, thematic maps on 1:12,500 scale are being prepared. These maps are used in preparation of development plans in both urban and rural sectors. The availability of 5.8 m multi-spectral data from IRS-P6, launched by ISRO in October 2003 and sub-meter panchromatic data, will enable the planners and resource managers to use them at micro level planning/ implementation level (1:5000 scale) specially in watershed development, waste land development, rural road connectivity, village level planning, site suitability etc. In urban sector, it will help in preparation of zonal plans and its monitoring, identification of waste disposal sites, infrastructure and utility planning etc.

In the field of terrain and topographic mapping, the availability of 2.5 m/1m PAN stereo data from Cartosat 1 and 2, in the near future, will help preparation of large scale (1:10,000) base maps/topographic maps.

Air-borne Laser Terrain Mapping System (ALTM) is another source of terrain data (DEM). ALTM provides DEM with 15 – 20 cm accuracy and thus makes it possible to prepare 0.5 m contour interval maps required in a variety of applications, both in rural and urban sector as well as in disaster management.

Very high resolution monoscopic data from the present and future earth observation satellites along with the high precision DEM from ALTM will open a new era of applications at micro level planning related to utility and infrastruc-

ture, alignment of communication network, pipe line routing, micro-watershed development, wetland conservation and habitat suitability, disaster management and impact assessment etc.

A large number of high resolution earth observation satellites are scheduled to be launched during the next decade and thus the mapping and cartographic discipline is going to see a major paradigm shift to meet the challenges, specially, in terms of better turn around time needed in mapping and surveying required for management and planning.

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Scientific research in Indian Universities : A downward trend

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What impedes scientific research in India? This is complex issue. The related questions are also difficult to answer. What has happened to Indian Universities which are supposed to be autonomous? Why the presence of mega-scientific research establishments, created by successive governments, is not being felt and appreciated by the common man? Apparently the funding to the mega-scientific establishments (like space, Atomic Energy, Defence, CSIR, IARI etc.) has increased over the years. The elite institutions like IISc, TIFR, IIT's etc have also managed to take a major share of whatever is left after the mega-establishments. This looks "rosy", particularly because these self-proclaimed best research establishments of yester-years of Indian Science are possibly becoming better in the eyes of a select few who matter. *Is it true?* The fact about overall research scenario in India is very disturbing. In terms of "quantity" of world scientific research, India ranked 8th during 1980"s. It slipped to 12th position in 1990"s and nose dived to the 21st position in the new millennium. When we introduce a measure of "quality" (science citation index, SCI) India's rank drops to dismal 119(see H.S. Virk, IAPT Bulletin 21,p.151-153,2004).How can this happen if all the adequately supported "elite and mega-establishments" were doing as well as claimed? *Possibly, there was another player in the arena of research in India which was a significant contributor to the research in India and which has now ceased to be so.* This player can easily be identified as "Universities". These are the much frowned upon and downgraded institutions by the scientific elite of today. Can we allow this to happen? If yes, the results could be more disastrous in future. What we are seeing now is just the beginning of the end of once flourishing Indian Science.

A case for encouraging research in universities :

Will encouraging research in the Universities be really fruitful? The answer could be "Yes" or "No" depending upon the perception of an individual or the line of argument adopted thereon. We have to be careful. Without negating the role of mega establishments and elite institutions, one must consider a subtle point that *the "method" of doing good science can possibly be learnt best in the University environment.* This is where lies the role of University research in the overall scenario of scientific research in the country. Let me elaborate the above stated philosophy or thought. Research is not only based on

"talent" but it is also an attitude, aptitude and state of mind. Developing and orienting the last three of these factors in young men, coming from different social background, can best be done in Indian Universities where student-student and student-teacher interactions are less formal and more personalized and where *teacher's "compassion" may create a "passion" to create something new and/or achieve something.*

There are numerous factors which distinguish research in Universities and mega institutions. Their **"goals and anticipated products"** are different. University's goal is to produce *creatively trained manpower for future while mega-establishments aim at specific research products.* One factor in favour of encouraging University research is discussed in the above paragraph. Another factor is easy to appreciate and discussed below. In a blooming University research environment, the young B.Sc. and M.Sc. students (age group 18-22 years) see the beaming faces of their teachers (which is doubtful today). That "shine" leaves an impression on them and encourages them to be creative. Frustrated faces will do the reverse. The teachers involved in cutting-edge research activity, are best exponents of current scientific thoughts even if their research is not the Nobel Prize winning material. Therefore, creation of young motivated researchers for the future becomes the direct outcome of a model research-orientation in the Universities. These will be the **ideal places to act as feeders** for the mega-institutions. This would take care of the complaints of the mega-research laboratories that the present day Universities are not able to supply the properly motivated and trained youths for research. This failure has been viewed as failure of the Universities but the blame is to be mostly or partly shared by the funding /controlling institutions which miserably fell short in appreciating the problems of *University Research starving for meager funding.*

The real output of the University research is " researcher and scientific leader of the future" and not research papers, products, devices or technologies. These do come in Universities but as a by-product of the process of University research. A student entering a University research programme is raw and made to face always an open-ended problem (I am distinguishing open-ended problem and ill-defined problem. The former is a challenge and the latter is a malaise introduced by some research supervisors and University rules, both of these are to be condemned.). The research supervisor in each case of a new student faces the daunting task of starting ab-initio and his duty is to raise him to the highest level of his/ her ability. Young University researchers, after attaining this, are ready to fly in the open skies. Their "initial exercise" in flying high in the open skies results in the research papers emanating out of the University research system. The point to be noted is that the flight of the youngman "*peaks only after he has left the University*". Will it not be naïve not to accept the role of Universities while passing judgment on freshly trained such researchers while always benefiting from their "peaks of achievement" in times to come. One has to judge and plan University research in this backdrop.

Perhaps in the above paragraph, I have raised the issue in an involved manner. Let me look it in a historical perspective which may be illustrative. In 1950's and early 1960's, there were very few University research centers. There were isolated

brilliant individual researchers in these Universities. The number of research papers were also few but those were full of new ideas. The University system was busy producing individuals who could successfully take up the real challenges in life, including research. Therefore, in all fields of national activity, the presence of these quality Universities was felt. These were ready to supply quality human resource to the few national laboratories, mega-research establishments, IIT's etc. All these started as Excellent centers. Then came the period of late 1960's and 1970's. The research/higher education scenario started becoming topsy-tervy. Mushrooming of big laboratories began with half-trained manpower (due to non-availability). A large number of Universities and Colleges sprang up, mostly under political pressures. These institutions were ill equipped in terms of proper teachers and facilities. Soon such institutions overshadowed the older institutions. So, the supply of good young researchers started dwindling. Universities started loosing respect. On the funding side, the national laboratories and mega-establishments took full control. They could do so because these were institutions manned and headed by individuals trained in hay days of the universities i.e. 1950's and early 1960's who were creatively peaking as explained in the earlier paragraph. In the race of high grade creative research, some University teachers did continue their race with the help of their earlier students (now heading the mega-establishments) and of course with the help of young fresh graduates. Initially, they did compete successfully. However, in 1980's race became a competition between two *"unequally nourished" players.* In 1990's and onwards, the low in university research became a talk of gossip and ridicule. I may point out that in 1970's, apart from University Intellectual Giants, many other University teachers did try to join the race but dropped out in frustration. Some continued the race with "dubious means". All this resulted in the shift of University research attitude from training good researchers to publishing "many" research papers to compete for more earthly "rewards" which was becoming order of the day. In this way, *University research was forced to go beserk.* We are seeing the negative results of this historical development. **Danger signal is on.** The scientific leaders of yester-years in the laboratories are aging and retiring. Their number is dwindling with no worthwhile back up. The Universities have lost respect which, coupled with poor research fund planning at the national level, is leading the young postgraduates to develop negative attitude towards research in general. This **trend has to be reversed** unless we all want to feel glorified in our role as "complaint boxes" rather than to take the path of " struggle for a better future".

The researchers in the Universities and the elite institutions groomed in 1960's have either retired or retiring soon. Unless something drastic is done to improve the Universities (which in turn would do good to other national laboratories), even the best of "optimist" will find hard to believe in the bright future of Indian Scientific research.

Where have we gone wrong?

Doers are to be considered as having wronged if a wrong has been committed. Therefore, it has to the University system, University teachers and students. This is a ridiculous conclusion and can, at best, be taken as a result of superficial thinking. In the present scenario, all the authorities

vested in Universities have been usurped by State/Central governments/UGC. The universities have no effective control on their own affairs. The visible control is " deceptively cosmetic". Teachers have been sidelined. Students have themselves chose to have no positive role and their role is getting limited to counterproductive "political" role. Therefore, the malaise, responsible for downward trend in university research, has its roots somewhere else. The social wings, which are directly or indirectly being nurtured by the Universities, have somehow, shaped themselves in a manner detrimental to the growth of creative research in universities.

Broadly, the following factors can be identified which are responsible for the neglect and downward trend of University research in India: -

I. Unimaginative democracy and the government at large

II. Society's perception of University research

III. Non-supportive mega-institutions

IV. Various constituents of the University system

These are **'EXPLOSIVES"** and may hurt the feelings of many. I have purposely raised these because we, as Indians, presently avoid speaking the truth for fear of revengeful, angry or unpleasant reactions. However, I am sure that many would see some merit in the above stated reasons for "malaise" after their initial anger settles down and they have read the following text as well.

Indian democracy is being run on fulfilling the short term goals and aspirations of the half-literate majority of electorates. *Investments in higher education and research give returns (positive or negative) after 10-15 years.* Investments in primary/secondary education are a shade better in this respect. The elections are held every five years. Therefore, the politicians tend to fail to find any visible effect of the investment in the higher education worth projecting before their electorates. Therefore, **democratically elected unimaginative governments of developing countries (with a poor literacy percentage) often play passive roles** in the development of higher education and research, which becomes a " casualty" of this callous attitude. Political bosses are only "proactive" when it comes to pushing their men and ideology for direct or indirect benefits. We all know that changes in the acts/statutes/ordinances of the universities are occasionally brought by Governments essentially to serve this purpose. What we are seeing today is the cumulative effect of deeds/misdeeds of one government after the other. Therefore, it is the enlightened electorates who have to speak out in the interest of the future generations of their own countrymen. If they do so, then *the derailed government policies may come back on rails.* Universities are, then, likely to again become places generating" light of wisdom" as well as creating "torch-bearers" to participate in the long run of national progress.

Not only the elected wings of the government, the executive wing of the government (so-called bureaucracy) is equally insensitive to the requirements of scientific research in the Universities. Possibly many executives would not believe and agree that they have failed to identify and designate specific funds for S & T research on a regular basis in the universities. Whatever we see are "doles" occasionally thrown. Interestingly, only

premier universities are considered fit to receive these "doles" under different names viz. Center of Advanced studies or CAS funds, Funds foe Infrastructure development, FIST etc .Of course, individual researchers can get project funds from DST,ICMR,ICAR,MNES,DOB,DAE,CSIR etc. These researchers have to be brave enough to bear the onslaught of the financial rules of their own universities, funding agencies, Audit etc. Nonetheless, such funds did help some enterprising teachers to keep the lamp of learning still burning in their respective areas of research activity.

Science Policy in India, which evolved since the time of Pt. Nehru, focused on the growth of mega-research establishments both in terms of administrative and actual laboratory infrastructures like DST, CSIR, Space, DAE etc. These made welcome contributions to start with. Now umpteen number of laboratories and establishments are mushrooming. All apparently are being created for furthering the cause of research in India. There is an " undercurrent" which blames that many such institutions were created to serve the "self" of the resourceful. Such opinions are person-based which may or may not be correct. The fact is that India today has many mega-establishments and we have to look at their relationships with the Universities. All these establishments need trained man-power and Universities are supposed to supply these. *Direct beneficiary of the universities are the mega-establishments.* Therefore, their support to the universities are expected. Is it happening or not? Anybody's guess! The support received by the universities from such establishments is "peanuts" Some feel that many mega-institutions act as " parasites". These institutions need to change their attitude. They shall feel obliged to help rather than obliging by helping. Over the past few years, the "morale" of University researchers has gone down because they were forced " *to beg at so many doors" for meager funds.*

Recruitment policies of most research institutions are proving to be non-conducive for attracting young minds to join university stream for research. These need to be reconsidered and revamped so that the trained Ph.D.'s find avenues. Message has gone " loud and clear" to youngsters that laboratories mostly recruit M.Sc.'s as Scientist B and are almost assured to reach to Scientist F on even G level. Opening for Ph.D.'s are few. And so why go in for Ph.D.!! Most science leaders of today were Ph.D.'s from Indian or Foreign Universities at the time of recruitment. So, they had the width and the wisdom, which most leaders of tomorrow will lack under the present recruitment/ promotion policy. The success of BARC Training School is generally referred to in the support of recruiting non-Ph.D.'s. It is an out of context reference. BARC has a specific job to carry out (not open-ended research) and for that they " train and teach". I again re-emphasize that for broad based training in creativity, Universities are the best place. Their role must be returned back to them except for very special cases. *Allow the universities to shine for turning out young faces shining with the brilliance of their research so that the overall national research may globally establish its shine.* Sometimes it may mean reaching out by the mega-institutions to the Universities rather than the reverse.

All constituents of the University system, including UGC, have to take major blame of their own downfall. From the

statements made in the earlier paragraphs, nobody should walk away with the impression that University functioning and callous attitude of the teachers can be condoned. NO, never! Had the teachers followed the path expected of a real academician (instead of engaging themselves in mud-slinging and filthy politics) and Universities exercised due control on the quality of their product, then it would have not been possible for any government/establishment/individual to ignore them.UGC's role is that of apathy and only as an on-looker. Let me take only one issue of recognition of any University/College for allowing it to run a research degree programme. If a Science Department is opened for a certain number of students for M.Sc., then University and the UGC ensures a certain number of properly qualified teachers, equipments and annual maintenance grant etc. For research, it is a free for all playground. There is no control or regular funding from UGC or Universities. Disgusting!

Teacher's behaviour has also resulted in a great damage. Exercising control on their personal behaviour and attitude is necessary. If not done by the teachers themselves, it has to be ensured the hard way by putting barriers in the promotion etc. There are teachers who distribute worthless Ph.D.'s in two years as "favours". Then there are teachers who exploit young students and take 6-8 years of student's valuable time before allowing the thesis to be submitted for their " self-grandiose" on the plea of quality. There are teachers who harass and humiliate the students which sends a negative message to their junior students who, in turn, start hating research as a career. It is fortunate, however, that there are still some teachers with "compassion and integrity" from whom we have some hopes. University Academic/administrative bodies (including UGC) have to find means to reward & encourage such teachers, while those who indulge in malpractices must face some reprimand. Control on teachers performance and their answerability has to be generated before we think of bringing back the glory of University research. The minimum basic requirement for this is appointment of a *proper Vice-Chancellor by the Government.* Somebody has to raise this issue and bell the cat!

The merit promotion scheme for teachers has served as the last nail in the coffin of University research. Merit promotion scheme was originally conceived to remove frustration of well deserving researchers in the University system. As practiced today, *doers and non-doers are treated as equals. In fact, the latter are generally treated "more than equal"* because they have more time to play politics and please local bosses. The story of time-bound promotions has roots in the promotion policy followed for government administrative posts .Then it entered in a modified way in Research laboratories. Universities followed this practice with a bang. Evil effects of neglecting research while considering promotions has started showing up. It needs to be arrested at all cost (ignoring the unpleasant reactions of teachers) for the long term benefit of the youth and future national development.

Concluding remarks

The downward trend of University research has been identified in this article. All concerned (including government, bureaucracy, scientific mega-establishments, UGC, University system, the teachers, the socio-political environment) stand to be

blamed collectively. Many points have been raised in this article which are likely to generate "controversy" and hence "discussions". If the latter happens, then the purpose of this article is served. I am aware that *generalities of the problem* have only been raised. Some specific malaise and possible remedies have also been indicated but these will be dealt later in another article. **University science is dying but not dead yet.** It has to be revived with vigour and commitment in the interest of the nation. *Let us rise now or else, it would be too late.*

Aflatoxin induced changes in protein and nucleic acid contents of germinating mustard seeds (variety Varuna)

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Abstract

Five different concentrations (viz., 100, 250, 500, 1000 and 2000 μg/l) of aflatoxin B_1 significantly lowered the levels of nucleic acids (DNA and RNA) as well as protein (both quantitative and qualitative) in germinating mustard seeds (variety Varuna). The extent of inhibition depended on the concentration of toxin treatment.

(Keywords : mustard seeds / aflatoxin B_1 / protein / DNA and RNA).

Introduction : Among oil seeds, mustard (*Brassica juncea* L.) is one of the most important crop in Bihar. Its oil is the main cooking medium in this part of the country. Besides adding a special flavour and palatability to food, it also acts as a lubricating agent to body tissue.

Like other oil-seeds, mustard seeds also harbour several mycotoxin producing fungi. The seeds have also been reported to be contaminated with aflatoxins and other mycotoxins during storage[1,2]. These mycotoxins have been found to influence various physiological and biochemical processes of crop plants. Since protein and nucleic acids are the important components of any seed, their synthesis has also been influenced by the presence of mycotoxins in the seeds[1,3].

An attempt has been made in this part of investigation to record the influence of aflatoxin B_1 on the syntheses of protein and nucleic acid during seed germination of mustard crop. Besides recording the levels of protein and nucleic acid, qualitative analysis of protein was also done by gel electrophoresis.

Materials and Methods : Seeds of mustard (variety Varuna) were obtained from the Oil-seed Division, Rajendra Agiculture College, Sabour. A stock solution of aflatoxin B_1 (Sigma, St. Louis, Missouri, USA) was prepared in 1 ml ethanol from which the dilutions (100, 250, 500, 1000 and 2000 μg/l) were made with distilled water. The seeds were steeped initially in distilled water for 1 h and subsequently in different concentrations of aflatoxin B_1 for 20h. For each treatment, 100 seeds were taken in triplicate. The steeped seeds were subsequently left for germination on moist blotting paper at 28±2°C. On seventh day quantitative and qualitiative estimations of the protein in germinated seeds were done by the spectrophotometric method[4] and the disc

electrophoretic method[5], respectively. The nucleic acid contents of the control and treated seeds were estimated by the method of Gottlieb and Tripathi[6]. The results were subjected to one way analysis of variance.

Results and Discussion : A significant decline in protein content of the seed was observed due to influence by different concentrations (100, 250, 500, 1000 and 2000 µg/l) of aflatoxin B_1 (Table 1). Per cent inhibitions in protein levels were 3.91, 6.61, 11.95, 21.01 and 43.36% at 100, 250, 500, 1000 and 2000 µg/l concentrations of aflatoxin B_1, respectively.

Protein quality as estimated through electrophorogram (Fig. 1) revealed the presence of 8 bands (4 major, 3 minor, 1 diffused) having different Rf values in germinated seeds. The quality of protein in the treated seeds was affected by aflatoxin B_1. It is apparent from Fig. 1 that at 100 µg/l concentration of aflatoxin B_1 maximum number of bands was noted. Band No. 9 appeared on the gel with the disappearance of band No. 3. Even at 250 µg/l concentration one new band (No. 10) appeared with the disappearance of 2 bands (No. 5 and 6). At 500 µg/l Band No. 9 disappeared and at the maximum

Table 1– Effect of different concentrations of aflatoxin B_1 on protein and nucleic acid (DNA and RNA) contents of mustard seeds.

Conc. of Afl. B_1(µg/l)	Protein content (µg/100ml)	DNA content (µg/100ml)	RNA content (µg/100ml)	% inhibitions in Protein	DNA	RNA
0	23.75 ± 0.35	14.06 ± 0.185	42.48 ± 0.386			
100	22.82 ± 0.19	13.84 ± 0.231	41.96 ± 0.185	3.91	1.56	1.22
250	22.16 ± 0.27	13.33 ± 0.172	39.67 ± 0.256	6.61	5.19	6.61
500	22.91 ± 0.30	12.28 ± 0.283	37.85 ± 0.238	11.95	12.66	10.89
1000	18.76 ± 0.22	10.41 ± 0.162	31.49 ± 0.362	21.01	25.96	25.87
2000	13.45 ± 0.12	7.80 ± 0.328	20.22 ± 0.769	43.36	44.52	52.40
t =	40.308	15.672	44.156			
r =	0.999	0.992	0.998			
df =	4	4	4			

concentration i.e. 2000 μg/l only 2 minor and 1 diffused bands remained in the seed.

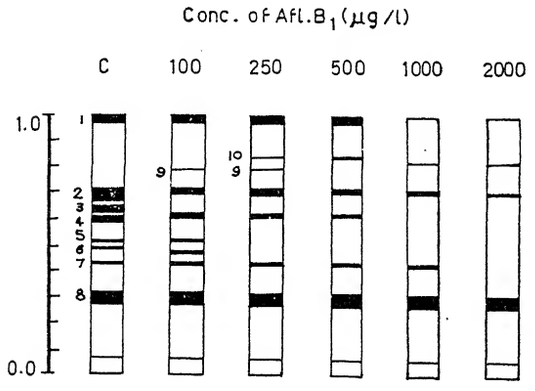

Fig. 1– Gel electrophorogram showing protein profiles of mustard seeds due to aflaxatoxin B_1 treatments

Aflatoxin B_1 was found to reduce drastically the number of bands of protein profile at different concentrations. At lower concentration some new bands were synthesized, which were found to be diffused at higher concentrations. The newly synthesized bands might be defensive one, acting against aflatoxin B_1. Electrophoretic variations in the seed protein due to mycotoxin have earlier been worked out in *Brassica* sp.[7], maize[8,9] and mung[10].

It is also evident from Table 1 that aflatoxin B_1 depleted nucleic acid contents (DNA and RNA) of mustard seeds. The total amount of DNA and RNA was measured as 14.06 and 42.48 μg/100 ml which was reduced to 7.80 and 20.22 μg/100 ml at higher concentration (2000 μg/l) of the toxin treatment. Inhibitions in DNA and RNA syntheses were also recorded at this concentration of aflatoxin B_1 in wheat (61.90 and 57.04%)[11], maize (58.11 and 53.55%)[9] and gram (56.79 and 51.26%)[1]. In fact when the toxin is introduced in the seeds at the time of germination it may interact with nucleic acids of the cells as reported in the case of animals. In animal cells aflatoxin B_1 undergoes biotransformation for its activation to convert into its epoxide forms which are cytotoxic as well as transformable because they can react with and oxidise DNA, RNA and protein constituents[12,13]. There are reports that the activated aflatoxin B_1 binds to guanine to form AFB_1 – DNA adduct[14]. Adducts are also formed in RNA[15] and protein molecules[13]. All the above processes ultimately lead to the reduction in further syntheses of DNA, RNA and protein[16]. Lillehoj and Ciegler[17] demonstrated remarkable effects of various levels of aflatoxin B_1 on the synthesis of DNA and RNA in *Flavobacterium aurantiacum*. They also observed that a concentration of 50 μg/l of aflatoxin B_1 completely blocked DNA synthesis in 4 hour incubation while reducing the RNA by less than 15% at that time.

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Formation of metal complexes of quercetin and its binding to calf thymus DNA

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Abstract

Metal chelating ability of quercetin with Fe^{2+} was studied using absorption spectroscopy. The binding constant was determined by following the absorption changes at 440 nm and also by following the kinetics of complexation. Fe^{2+}-quercetin complex showed binding to calf thymus DNA, as seen by the absorption spectral changes. Using pulse radiolysis technique, the one-electron oxidation of quercetin and Fe^{2+}-complex was found to be similar indicating that the active site undergoing oxidation is not affected by complexation with Fe^{2+}.

(Keywords : quercetin / Fe^{2+} complex / DNA binding / pulse radiolysis)

Introduction

Plant flavonoids are well known for their pharmacological activities. Quercetin is one of the most abundant bioactive flavonoids. It is a powerful antioxidant and exhibits anticancer and antiviral activity[1-4]. It is a known free radical scavenger. The antioxidant potential has been attributed due to the neutralization of the damaging effects of free radicals like reactive oxygen and nitrogen species[4-6] and also by preventing their production through chelation with the metals[7-9]. Metals play an important role in metal overloaded diseases and also in oxidative stress conditions[10]. Transition metal ions such as Fe^{2+} participate in Fenton reactions and generate harmful free radicals like hydroxyl radicals. Converting free metal ions into chelates can change the redox properties of the metals which can also modulate the reaction with hydrogen peroxide, thereby effecting the production of hydroxyl radicals[10]. With these objectives, the chelating property of quercetin with Fe^{2+} has been investigated using absorption spectroscopy and stopped-flow spectrometer. The binding of metal complexes to calf thymus DNA has also been studied. Effect of metal complexation on the one-electron oxidation of quercetin was studied using pulse radiolysis technique.

Materials and Method

Quercetin, calf thymus DNA and ferrous ammonium sulfate were obtained from

Sigma Chemicals, USA and used without any further purification. Spectrograde dimethyl sulfoxide (DMSO) and 'nanopure' water from Millipore A-10 system were used for preparing the solutions. Absorption spectra were recorded on a Hitachi spectrophotometer (model 330). Stopped-flow studies were carried out on SX-18 MV stopped-flow analyzer from Applied Photophysics, UK in single mixing mode. For pulse radiolysis studies, high energy electron pulses (7 MeV, 50 ns) were obtained from a linear electron accelerator whose details are given elsewhere[11-13]. One-electron oxidation studies were carried out with $Br_2^{\cdot-}$ radicals, generated on pulse radiolysis of N_2O-saturated aqueous solutions containing 0.1 M KBr and the details are given elsewhere[14].

Results and Discussion

Complex formation between quercetin and Fe^{2+}

5% aqueous DMSO solution of quercetin shows absorption spectrum in the wavelength range from 200 to 500 nm region with two absorption bands at 250 nm and at 365 nm (Fig. 1). Addition of Fe^{2+} solution to 5% aqueous DMSO solution of quercetin showed decreased absorption at 365 nm with simultaneous build up of a new band at 440 nm. The intensity of absorption band at 440 nm increased with increasing Fe^{2+} concentration from 0 to 100 μM (Fig. 1), suggesting the formation of complex between quercetin and Fe^{2+} (equation 1). However, no clear isosbestic point was observed although for low concentrations of Fe^{2+} (0 – 25 μM) an isosbestic point was seen at 400 nm.

$$\text{Que} + Fe^{2+} \rightleftharpoons \text{Complex} \quad (1)$$

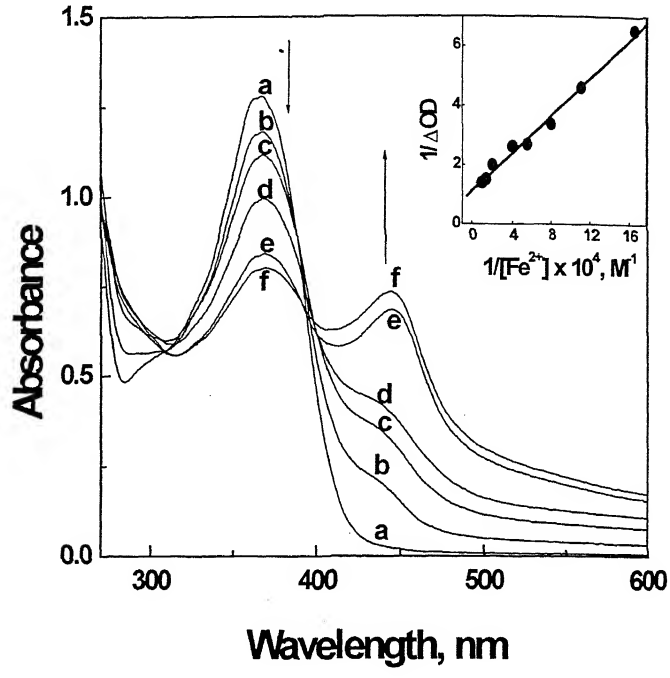


Fig. 1– Optical absorption spectra of 25 μM quercetin in presence of different concentrations of Fe^{2+}; a–0; b–9; c–18; d–25; e – 75 and f–100 μM. Inset shows Benesi-Hildebrand plot for Fe^{2+}complexation with quercetin.

The equilibrium constant (K) for 1:1 complex was determined by employing Benesi-Hildebrand equation (2)[15].

$$\frac{1}{\Delta A} = \frac{1}{K\Delta\varepsilon_{440}[\text{Que}][Fe^{2+}]} + \frac{1}{\Delta\varepsilon_{440}[\text{Que}]} \quad (2)$$

Here ΔA is the increase in the absorbance at 440 nm and $\Delta\varepsilon$ is the change in molar extinction coefficient at 440 nm. The absorption changes at 440 nm were followed as a function of Fe^{2+} concentration at a constant quercetin concentration (25 μM). The plot of $1/\Delta A$ *vs* $1/[Fe^{2+}]$ gave a straight line (inset of Fig. 1) with slope = $1/K\Delta\varepsilon[\text{Que}]$ and intercept = $1/\Delta\varepsilon[\text{Que}]$.

From this, the equilibrium constant and molar extinction coefficient for 1:1 complex were determined to be 1.5 x 10^4 M^{-1} and 6.1 x 10^3 M^{-1} cm^{-1} respectively. A good linear relationship (inset of Fig. 1) support the formation of 1:1 complex in low concentration region. However, in the higher concentration region (> 100 μM) it deviated from linearity suggesting possible formation of complexes of different stoichiometry.

Kinetics of complex formation using stopped-flow spectrometer

The equilibrium constant (K) for the binding of quercetin with Fe^{2+} was also determined by following the kinetics of complex formation using stopped-flow kinetic spectrometer. For this the two mixing syringes contained 10 μM Fe^{2+} and varying concentration of quercetin (25-100 μM) separately. The absorption changes were monitored at 440 nm over a period of 20 s. The absorption-time profile (inset of Fig. 2) was fitted to an exponential function and the rate constant was determined. This observed rate constant (k_{obs}) varied with the forward and backward rate constants as follows:

$$k_{obs} = k_f\{[Que] + [Fe^{2+}]\} + k_b \quad (3)$$

where k_f and k_b are the forward and backward rate constants and the equilibrium constant $K = k_f/k_b$. According to the above equation, a linear plot was obtained for the variation of k_{obs} as a function of equilibrium concentration of both quercetin and Fe^{2+} (Fig. 2). The slope and intercept of the linear plot gave the values of k_f and k_b respectively. The equilibrium constant for 1:1 complex was evaluated to be 1.34 x 10^4 M^{-1}. This value is close to that obtained by the steady state absorption methods. The quercetin-Fe^{2+} complexation can take place at the catechol site or flavone ring. These two binding sites are shown in Scheme 1.

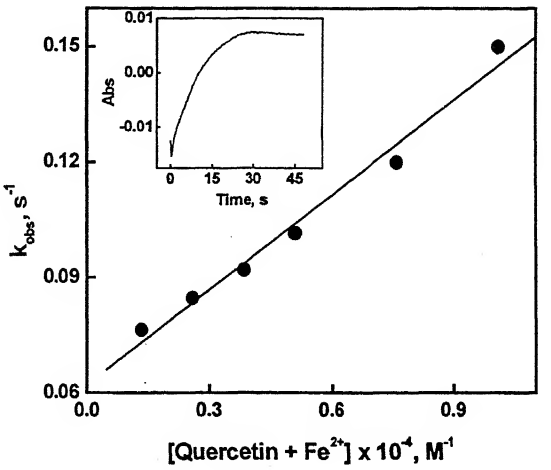
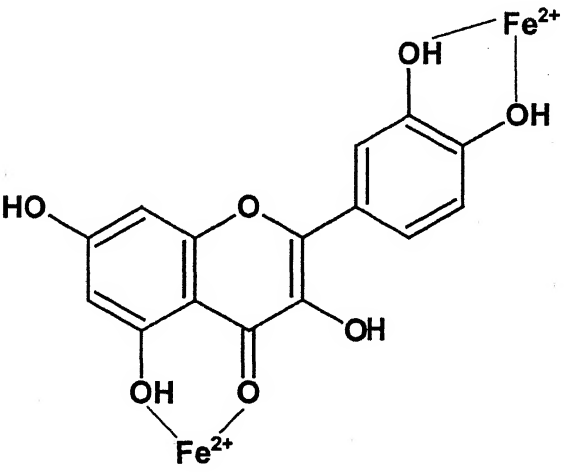


Fig. 2– The variation of pseudo-first order rate constant (k_{obs}) for the formation of Fe^{2+} quercetin complex (λ = 440 nm) on mixing 20 μM Fe^{2+} and different concentrations of quercetin (50-200 μM). Inset shows absorption-time profile at 440 nm.



Scheme 1

Binding of Fe^{2+}-quercetin complex to DNA

The absorption spectrum of quercetin did not show any change on addition of calf thymus DNA solution (up to 100 μM). However, the absorption spectrum of Fe^{2+}-quercetin complex showed increase in absorption at 430 nm on addition of DNA (Fig. 3) suggesting the binding of DNA to quercetin-Fe^{2+} complex (equation 4).

$$\text{Que–Fe+DNA} \rightleftharpoons \text{Complex} \quad (4)$$

The equilibrium constant for the binding of DNA to complex was determined by monitoring the absorbance changes at 430 nm as a function of DNA concentration. The linear plot of $1/\Delta A$ *vs.* 1/[DNA] (inset of Fig. 3) gave binding constant value equal to 3.4×10^4 M^{-1}. This suggests that the Fe^{2+}-quercetin complex can bind to DNA possibly through the electrostatic interaction between positively charged quercetin-Fe^{2+} complex and negatively charged DNA.

One-electron oxidation of quercetin-Fe^{2+} complex

One electron oxidation studies of quercetin and quercetin-Fe^{2+} complex were carried out with pulse radiolytically generated $Br_2^{\cdot-}$ radicals at pH 7. $Br_2^{\cdot-}$ radicals are specific one-electron *oxidants* with a potential of 1.6 V *vs.* NHE[14,16]. The oxidation of quercetin, by pulse radiolysis, has been reported where a transient spectrum with two absorption maxima at 300 and 550 nm was observed[5]. The transient spectrum has been attributed to

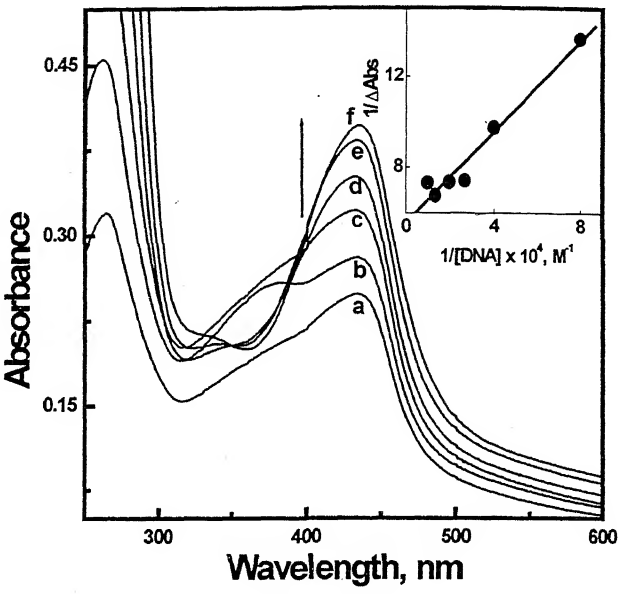


Fig. 3– Optical absorption spectra of Fe^{2+} quercetin complex (Fe^{2+} = 12.5 μM; quercetin = 25 μM) containing different concentrations of calf-thymus DNA; a – 0; b – 6; c – 9; d – 25; e – 50 and f - 100 μM. Inset shows Benesi-Hildebrand plot for binding of DNA with Fe^{2+} -quercetin complex (λ = 430 nm).

the oxidation at the 3', 4' hydroxyl groups (catechol site) of quercetin producing the corresponding phenoxyl radicals[5]. In order to understand the nature of oxidation of the Fe^{2+}-quercetin complex, $Br_2^{\cdot-}$ radicals reaction was studied with quercetin and its 1:1 complex. The nature of the transient spectrum (Fig. 4) remained unchanged in both quercetin and its complex, indicating that the possible site of attack for the oxidation is the same in both quercetin and its Fe^{2+}-complex. These studies suggest that catechol site where oxidation is taking place is unaffected by complexation. Therefore, complexation of quercetin with Fe^{2+} does not alter its free radical induced oxidation reactions.

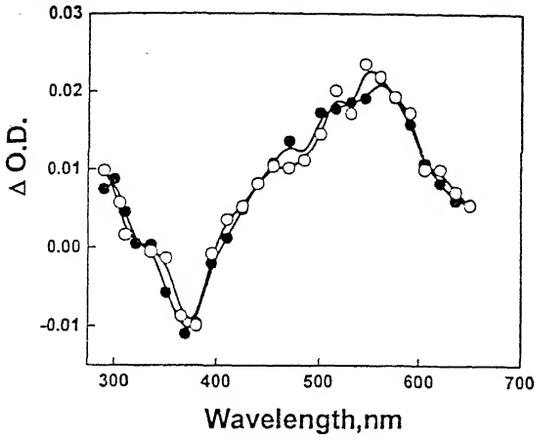


Fig. 4– Difference transient optical absorption spectra obtained on pulse radiolysis N_2O-saturated aqueous solution of KBr (0.1 M) containing 50 μM quercetin – (•) and Fe^{2+}-quercetin complex (Fe^{2+} = 25 μM; quercetin = 50 μM) – (o) 200 μs after the pulse. Dose = 6.8 Gy per pulse.

Conclusions

Quercetin, a well-known flavonoid antioxidant forms chelates with Fe^{2+}, with a stoichiometry of 1:1. The binding constant for the complex formation was found to be of the order of 10^4 M^{-1}, determined by both absorption and stopped-flow kinetic studies. The 1:1 complex shows selective binding to DNA. The one-electron oxidation reaction of the complex was found to be similar to the uncomplexed quercetin. Based on these results it can be concluded that metal complexation of quercetin is one of the responsible factors for its antioxidant activity. Since metal complexes possess binding to DNA, it is possible to modulate the site-specific antioxidant power of quercetin by complexing with metals like Fe^{2+}.

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Effect of external electric field on critical parameters and equivalence between the electric field and supersaturation ratio during water vapour condensation and ice glaciation

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Abstract

In presence of an external electric field water droplets have been found to grow with time. Theory of droplet growth in presence of external electric field has been applied to nucleation phenomenon of water vapour condensation and ice glaciation. The phase change depends on temperature and supersaturation ratio.

In the present study, the radius of critical nucleus, number of water molecules in it and Gibbs' free energy of its formation have been calculated as the function of temperature and supersaturation ratio.

These parameters have also been calculated in presence of external electric field as the function of electric field and relaxation time. The parameters in presence of electric field have been found much less than those obtained in electric field free case, but the equilibrium concentration of nuclei is very much larger. The equivalence between external electric field and supersaturation ratio suggests that small value of electric field is equivalent to very high supersaturation ratio to get nucleus of given size under similar conditions of temperature.

(Keywords: supersaturation ratio/nucleation/ Gibbs' free energy/ relaxation time/ electric field).

Introduction

It is a well known fact that there exists a vertical electric field in free and undisturbed atmosphere. Also, intense electric fields are generated in thunderclouds[1]. The intense electric fields in thunderclouds are not widespread and charge and intense electric fields seem to be concentrated in relatively small volumes, although, under actual cloud conditions the electric field varies considerably and its rate of growth has been calculated[2].

In general, during lightning the electric field is produced near lighting channel. The electric field so generated affects the rate of nucleation. Moore *et al.*[3] and Levin and Ziv[4] reported a rain gush following lightning stroke. During a lightning discharge maximum electric field (~10 e.s.u.) is produced near the channel. Thus, the electric field plays an important role in cloud physics. This role could be effectively verified in absence of saturation.

Murino[5] has shown that the action of a constant and uniform electric field accelerated the condensation of water vapour by a factor depending upon the intensity of electric field. It was shown that a droplet acquires a particular size under very low supersaturation under an electric field, which would, otherwise, require very high supersaturation. He discussed the polarization of water vapour molecules in the electric field of the central dipole (embryo of water) alone. Recently, Singh *et al.*[6] modified the value of polarizabilities by including the, effect of transnational and rotational motion.

Singh *et al.*[7] have shown, that in the resultant effect on a droplet due to an external electric field and the field induced due to central dipole, rate of nucleation in water vapour condensation and ice glaciation is about 100 times more near breakdown for dry air, as compared to that in absence of electric field. It has been shown[8-14] that the electric field induces the nucleation in liquid. Evans[15] demonstrated experimentally the effects of electric field on the production of ice crystals in cloud chambers and argued that the accelerated charged water molecules move to crystal tips, thereby increasing the nucleation rate. Avila *et al.*[16] gave the effect of cloud droplet spectrum on electrically charge transfer during individual ice-ice collision. Microphysical growth state of ice particles and large-scale electrical structure have been given by Williams *et al.*[17]. Saunders[18] have serious comments on it.

Following kinetic theory, Collins[19] inferred that the relaxation time is independent of free energy of formation of the nucleus, but it varies as the square of the radius of the critical nucleus.

In the present study we have considered the resultant effect due to an external electric field and the induced electric field due to the central dipole. These theoretical considerations are applied to the nucleation process in water vapour condensation and ice glaciation in order to estimate the critical size of the nucleus. This shows that the critical nucleus is attained in a time less than in electric field free nucleation. In the light of the present modification the effect of an electric field on the relaxation time *via* the calculation of critical size of nucleus in homogeneous nucleation is considered. The comparison with homogeneous nudeation for relaxation times and the rate of nucleation reveals that the fields even in electrically active clouds are insufficient to make the homogeneous process as effective as the heterogeneous process. Further, the equivalence has been made between electric field and supersaturation ratio.

Theoretical Consideration

Water vapour is present in atmosphere. Under suitable conditions of temperature and supersaturation, phase change takes place. The water exists in all three phases: vapour, liquid and solid. Thus there may be any of the following three changes:

(a) Water vapour $\xrightarrow{\text{condensation}}$ liquid water

(b) Water vapour $\xrightarrow{\text{condensation}}$ liquid water $\xrightarrow{\text{freezing}}$ solid water

(c) Water vapour $\xrightarrow{\text{glaciation}}$ solid water (ice)

The frequency of growth of embryos beyond a critical size in the nucleation process corresponds to a free energy maximum. This energy is

$$\Delta G_w = -(\mu_v - \mu_w) n_w + \sigma_{w/v} S_n \quad (1)$$

where μ_v is the chemical potential of vapour; μ_w the chemical potential of water; $\sigma_{w/v}$ the surface tension of water-vapour interface; S_n the surface area of the embryo and n_w the number of water molecules in water embryo.

Eqn. (1) may be written as

$$\Delta G_w = -(4/3)\Pi r_w^3 \Delta G_v + 4\Pi r_w^2 \sigma_{w/v} \quad (2)$$

where, ΔG_v is the value of Gibb's free energy of the condensate per unit volume per mole, with

$$\Delta G_v = (\rho_w RT/M_w) \ln S_{v.w} \quad (3)$$

where r_w is the radius of water nucleus; ρ_w density of water; T the temperature; M_w the molecular weight of water; R the universal gas constant and $S_{v.w}$ the supersaturation ratio of vapour over water surface.

Corresponding to the maximum free energy of the critical size of the nucleus, we have

$$\partial \Delta G_w / \partial r_w = 0 \quad (4)$$

which yields the radius of critical nucleus

$$r_w^* = 2M\sigma_{w/v} / \sigma_w RT \ln S_{v.w} \quad (5)$$

and the number of water molecules in a critical nucleus is given by

$$r_w^* - 4\Pi r_w^{*3} \rho_w N / 3M_w \quad (6)$$

where N is Avogadro's number.

The energy of formation of critical nucleus becomes

$$\Delta G_w^* = (4/3) \Pi r_w^{*3} \sigma_{w/v} \quad (7)$$

and equilibrium concentration of critically sized nuclei is given by

$$C(n_w^*) = C(1)_0 \exp [-\Delta G_w^*/RT] \quad (8)$$

where $C(n_w^*)$ is the concentration of nuclei with n_w^* water molecules; $C(1)_0$ the concentration of monomers, and k the Boltzmann constant.

In presence of electric field

Water is a strongly polarizable medium. The external electric field induces an electric dipole moment on both the water droplet and surrounding water vapour. The rate of increase of droplet radius has been derived as

$$dr_w/dt = (\rho_v/\rho_w) (9\alpha\lambda E^2/m_w)^{1/2} \quad (9)$$

where λ is the mean free path of water molecules; E the external electric field; m_w the mass water vapour molecule; ρ_v the density of water vapour; α the polarizability; ρ_w density of water and r_w the radius of water nucleus in electric field.

Integrating eqn.(9) within limits $r_w = 0$ to $r_w = r_w^*$ (critical radius of the nucleus) and $t = 0$ to $t = \tau$ (relaxation time), we get

$$r_w^* = [3\rho_v \tau (9\alpha\lambda E^2/m_w)^{1/2}/2\rho_w]^{2/3} \quad (10)$$

Water Vapour -ice glaciation

The process (c) for phase change is equally described from eqn. (1-10), with the replacements:

$\mu_w \rightarrow \mu_i$, $\sigma_{w/v} \rightarrow \sigma_{i/v}$, $\Delta G_w \rightarrow \Delta G_i$,

$r_w \rightarrow r_i$, $r_w^* \rightarrow r_i^*$, $n_w \rightarrow n_i$,

$S_{v.w} \rightarrow S_{v.i}$, $\rho_w \rightarrow \rho_i$

Equivalence between electric field and supersaturation ratio

If the same size of nucleus is obtained in two cases: in absence and in presence of electric field, R.H.S. of eqn.(5) and (10) may be equated to get.

$$\frac{2M_w\sigma_{w/v}}{\rho_w RT \ln S_{v,w}} = \left[\frac{3\rho_v\tau(9\alpha\lambda E^2/m_w)^{1/2}}{2\rho_w}\right]^{2/3} \quad (11)$$

which reduces to

$$E_{eq} \ln S_{v.w} = [4M_w\sigma_{w/v}/3RT\rho_v] (m_w/9\alpha\lambda)^{1/2} \quad (12)$$

Eqn. (12) may be written as

$$E_{eq} \ln S_{v.w} = [K^{!}/T\tau] \quad (13)$$

where E_{eq} is the equivalent electric field and $K^{!}$ is a constant given by

$$K^{!} = [4M_w\sigma_{w/v}/3R\rho_v(m_w/9\alpha\lambda)^{1/2}] \quad (14)$$

For a given value of temperature and relaxation time, eqn. (13) becomes

$$E_{eq} \ln S_{v.w} = K^{!!} \quad (15)$$

where $K^{!!} = K^{!}/T\tau$

From eqn. (15) we have

$$S_{v.w} = \exp[K^{!!}/E_{eq}] \quad (16)$$

which shows that the supersaturation ratio $S_{v.w}$ varies exponentially with equivalent electric field E_{eq}.

Results and Discussion

In the present calculations the constant values used are:

$\lambda = 10^{-5}$cm, $\rho_v = 10^{-5}$ at 10°C, $\alpha = 5\times10^{-23}$ cm^3, $m_w = 3\times10^{-23}$ g, $R = 8.317\times10^7$ erg. K^{-1} mole^{-1}, $\rho_w = 1.0$, $\rho_i = 0.917$, $\sigma_{w/v} = 72$erg cm^{-2} and $\sigma_{i/v} = 100$ erg. cm^{-2}

The calculated values of critical ice radius, number of water molecules in a critical ice nucleus, Gibb' s free energy of formation of critical ice nucleus varying with supersaturation ratio as the function of temperature has been plotted in Fig. 1 (a, b, c). Similarly, variation of these parameters with electric field as the function of relaxation time τ has also been shown in Fig. 2 (a, b, c). From these figures it is concluded that at given temperature r_i^*, n_i^* and ΔG_i^* decrease with increase in supersaturation ratio (Fig. 1). The rate of decrease is faster for low values of the supersaturation ratio but it is slower for higher values of supersaturation ratio.

In the presence of electric field, as is obvious from Fig. 2 (a, b, c) the values of r_i^*, n_i^* and ΔG_i^* for a given relaxation time increase with increase in electric field. These are also found to increase with increase in relaxation time. The rate of increase is faster at low values of supersaturation ratio, while, it is slower at larger values of supersaturation ratios.

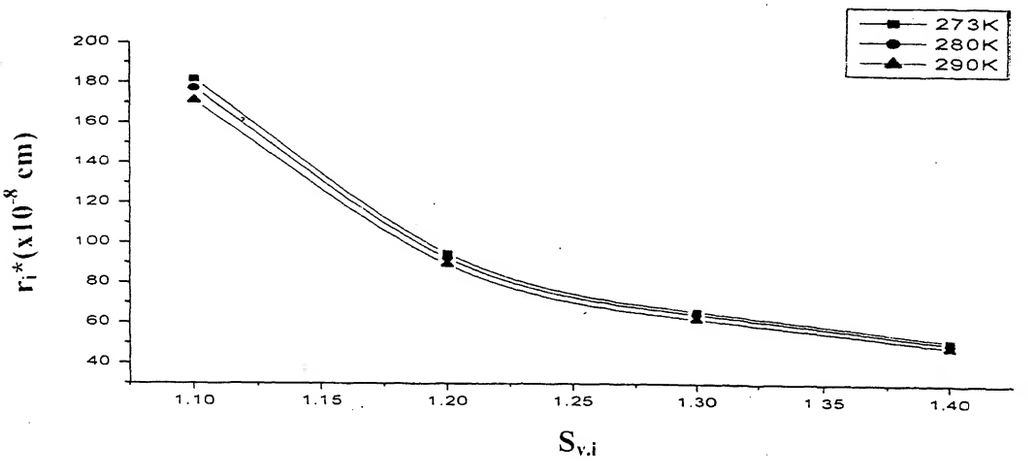

Fig. 1. a–Values of critical radius of ice nucleus r_i^* as the function of temperature and supersaturation ratio $S_{v.i}$ in absence of external electric field.

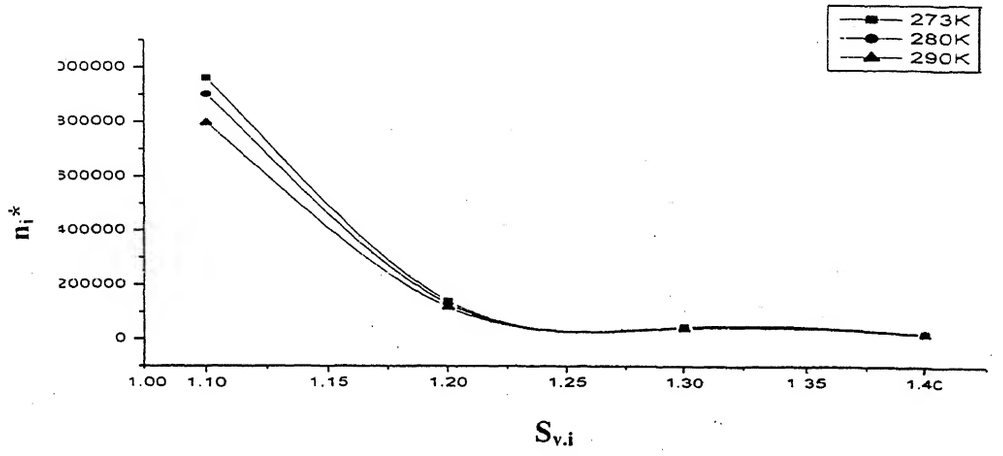

Fig. 1.b–Number of water molecules in a critical ice nucleus n_i^* as the function of temperature and supersaturation ratio $S_{v.i}$ in absence of external electric field.

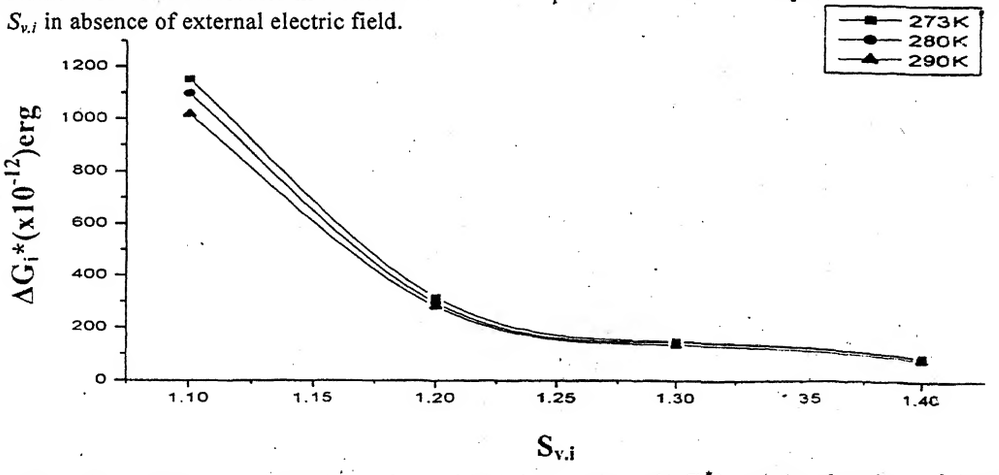

Fig. 1.c–Gibb's free energy of formation of a critical ice nucleus (ΔG_i^*) as the function of temperature and supersaturation ratio $S_{v.i}$ in absence of external electric field.

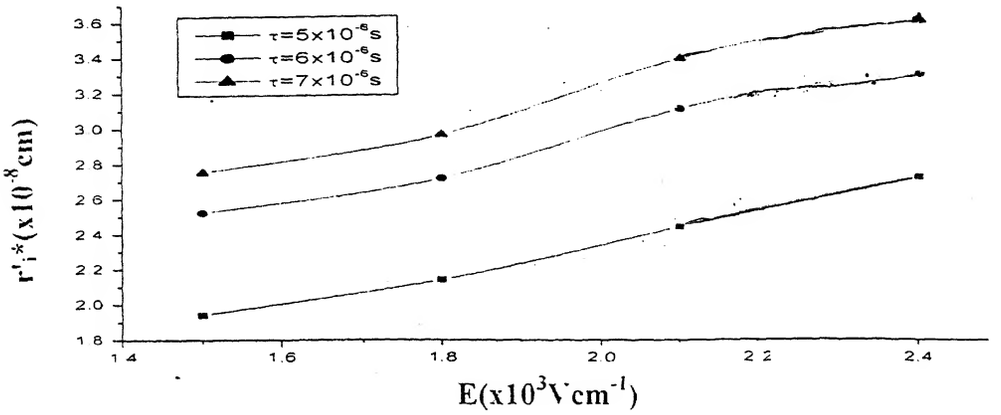

Fig. 2.a– Values of critical radius of ice nucleus r_i^* as the function of relaxation time (τ) and electric field (E) in presence of electric field.

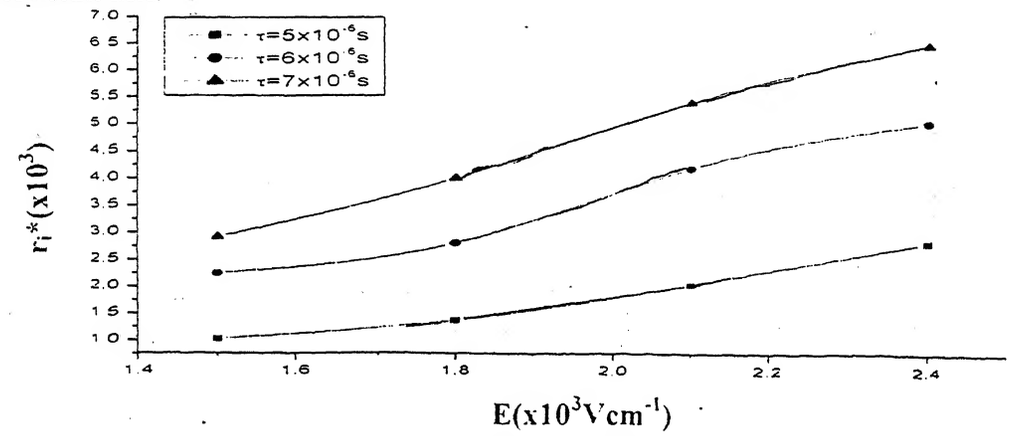

Fig. 2.b– Number of water molecules in a critical ice nucleus n_i^* as the function of relaxation time (τ) and electric field (E) in presence of electric field.

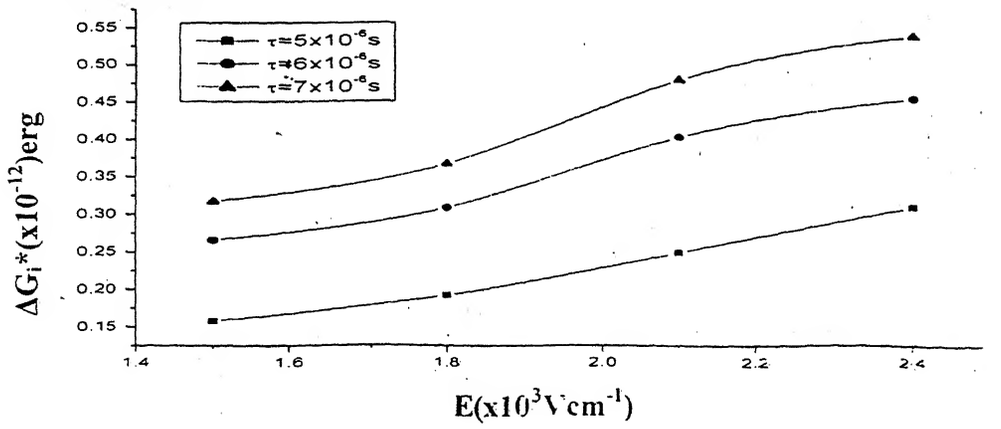

Fig. 2.c– Gibb's free energy of formation of a critical ice nucleus (ΔG_i^*) as the function of relaxation time (τ) and electric field (E) in presence of electric field.

The above theoretical calculations have been made with the assumption that at a particular altitude the value of electric field remains constant and uniform for long time unless the weather conditions of the cloud - atmosphere are changed.

Table 1 represents the equivalence of supersaturation ratio with the electric field in the case of water vapour condensation. The equivalence depends on temperature and relaxation time as well. For example, in the absence of electric field, critical nucleus of radius 119.78A° is formed at temperature 273K and at supersaturation ratio 1.1 using eqn.(5). The same size of the nucleus would have been obtained in presence of electric field of 142.72×10^3 Vcm^{-1} with relaxation time 5×10^{-6} s using eqn.(10). Thus, we note that supersaturation ratio 1.1 at 273K is equivalent to an electric field of 142.72×10^3 Vcm^{-1} with relaxation time 5×10^{-6} s. For given relaxation time and temperature the equivalent electric field decreases with increase in the supersaturation ratio and *vice versa*. For example with relaxation time 7×10^{-6} s and temperature 290K, the equivalent electric' fields are 101.95×10^3, 38.54×10^3 and 12.32×10^3 Vcm^{-1} corresponding to supersaturation radios 1.1,1.2 and 1.3, respectively.

At given temperature and supersaturation ratio, the equivalent electric field also decreases with increasing relaxation time.

At a given relaxation time corresponding to a supersaturation ratio, the equivalent electric field decreases with increase in temperature, e.g. for $\tau = 6 \times 10^{-6}$ s and $S_{v,w} = 1.1$, $E_q = 118.94 \times 10^3$, 114.50×10^3 and 108.69×10^3 Vcm^{-1} at T = 273K, 280K and 290K, respectively.

Conclusions

From the above study, it is concluded that the nucleation of water droplets and ice particles in electric field is very much effective. A small external electric field is equivalent to very large value of

Table 1– Values of E_{eq} corresponding to S_{vw} for given r_w * as the function of τ and T

r_w* (x10^{-8}cm) and E_{eq} (x$10^3$$Vcm^{-1}$) at temperatures

T	273 K		280K		290K	
$S_{v,w}$	r_w^*	E_{eq}	r_w^*	E_{eq}	r_w^*	E_{eq}
			$\tau=5\times10^{-6}$ s			
1.1	119.78	142.72	116.78	137.40	112.75	130.43
1.2	62.62	53.95	61.05	52.00	58.94	49.21
			$\tau=6\times10^{-6}$ s			
1.1	119.78	101.95	116.78	98.14	112.75	108.69
1.2	62.62	44.96	61.05	43.33	58.94	41.05
			$\tau=7\times10^{-6}$ s			
1.1	119.78	101.95	116.78	98.14	112.75	93.17
1.2	62.62	38.54	61.05	37.14	58.94	35.15

supersaturation ratio, which, otherwise, never exists in the clouds. Also in electric field induced nucleation the critical nuclei of smaller sizes are formed containing smaller number of water molecules and require less energy for formation. Hence equilibrium concentration of critical nuclei (water .and ice) is enhanced as compared to the electric field free case of nucleation. Also from the equivalence of supersaturation ratio with electric field, it is evident that electric field is more efficient than supersaturation. This explains the rain gush after lightning.

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Impact of monoamines on the spontaneous electrical activity in the scorpion, *Heterometrus fulvipes*

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Abstract

Effect of monoamines on the spontaneous electrical activity was studied in the ventral nerve cord of the scorpion, *Heterometrus fulvipes*. Perfusion of nerve cord with epinephrine, norepinephrine, dopamine and serotonin (5-HT) exerted significant inhibitory action on the spontaneous activity within 5 to 10 minutes. Washing of nerve cord with scorpion ringer, however relieved the effect and the activity showed recovery towards control levels. The results were discussed in relation to role of these monoamines in social and aggressive behaviour of scorpions.

(Keywords : monoamines/ scorpion/ electrical activity)

The anatomical details of the scorpion CNS have been extensively studied.[1-4] Subsequent studies on scorpion nervous system have been mostly confined to either biochemistry of cephalothoracic mass[4] or the electrical activity in the ventral nerve cord[5,6]. The pharmacology of the scorpion nervous system, however has not been explored and the role of different neurotransmitters other than ACh has not been looked into. There have been few studies on neurochemical parameters associated with behaviour in arthropods.[7-9] The modulatory effects of biogenic amines on olfactory receptor neurons have been studied.[10] Only studies available on these aspects in scorpions are from our laboratory[11] and.[12,13] Therefore, the present study was taken up to test the effect of various neurotransmitters (epinephrine, norepinephrine, dopamine and serotonin) on the spontaneous electrical activity in the ventral nerve cord of the scorpion *Heterometrus fulvipes*.

The scorpions (*Heterometrus fulvipes*) were collected from the nearby fields and were maintained in the laboratory in glass containers. The bottom of the containers was filled upto 10 cm height with soil collected from the field where scorpions were collected. The scorpions were exposed to natural day (24h light-dark cycles; 28±3° C). These were fed with cockroaches. Active and healthy adult scorpions were selected and tied on to a dissection board with the dorsal side up by means of rubber bands. The dorsal body wall was removed and the nerve cord (VNC) was exposed from cephalothoracic nerve mass (CTNM) to metasoma. The VNC was always kept immersed in scorpion ringer[14], during the period of dissection and experimentation. Recordings were made in air at room temperatures

(30-32° C). Before recording, the cord was allowed to remain in the ringer for at least 5 minutes. Spontaneous electrical activity of control animals was recorded for 30 minutes to confirm whether there was sustained activity throughout the period. The portion of the connective under which the electrodes were placed was covered with a drop of liquid paraffin to avoid the drying of the connective. Similar procedure was followed to test the effects of different neurotransmitters. Different concentrations of neurotransmitters (epinephrine, nor-epinephrine, dopamine and serotonin) ranging from 1 x 10^{-6}M to 1 x 10^{-3}M were prepared in scorpion ringer. Each transmitter solution was tested for its effect from lower concentration (1 x 10^{-6}M) to higher concentration (1 x 10^{-3}M) until there was a significant effect on the spontaneous activity. Since the spontaneous electrical activity was found to be greater in the connectives between the first and second ganglia, the same region was chosen for electrophysiological studies. The time of recording was around 20-00h at which time animal exhibits maximum activity in 24h day. During the first 10 minutes, fresh ringer was dripped into abdominal cavity at the rate of 3.5ml/min. After 10 minutes the saline reservoir was replaced with a container having saline with neurotransmitter to be tested and dripped for 10 minutes (3.5ml/min). After that, the ringer reservoir with neurotransmitter was replaced with a fresh ringer reservoir and dripped (3.5ml/min) for 10 minutes to note whether there was any recovery from the effect of neurotransmitter. Thus, the entire time of recording was 30 minutes. The signals from the VNC were amplified by P5 Grass AC preamplifier and fed simultaneously to Systronics 5100 dual beam oscilloscope and audio cassette recorder. Grass C4 kymograph camera was used to transfer the signals from oscilloscope to the photographic film. The tape recorded information was fed to the 4-digit counter to count the number of spikes per minute. The spike counts were also checked manually and presented as number of spikes/min.

The action of epinephrine (1 x 10^{-3}M) was found to be inhibitory on the spontaneous electrical activity of scorpion VNC. The frequency and amplitude of activity was decreased to the maximum (40.22%) at 15 minutes (5 minutes after perfusion). The decrease of the spontaneous activity was statistically significant ($P > 0.05$)(Fig. 1A & 1B). Recovery observed followed by ringer wash was not significant. At a concentration of 1 x 10^{-3}M, norepinephrine produced inhibitory action on the electrical activity of the VNC. Maximum decrease (37.66%) on the firing rate and amplitude of the spikes were observed at 6 minutes after the administration of norepinephrine. The large fibre activity which disappeared due to the inhibitory effect of norepinephrine appeared again after ringer wash (Fig. 2A & 2B). The inhibitory action of nor-epinephrine was statistically significant ($P > 0.05$). Dopamine, at a concentration of 1 x 10^{-3}M, inhibited the spontaneous electrical activity in the VNC. The inhibitory action was slow and took 6 minutes to cause maximum inhibition (36.34%). The spikes of high amplitude also disappeared. Few giant spikes appeared again after ringer wash and the activity in general showed an increase toward control levels with in 10 minutes after ringer wash (Fig. 3A & 3B). The monoamine serotonin, at a concentration of 1 x 10^{-3}M inhibited spontaneous activity of VNC. The inhibitory action was slow and the effect was maximum (43.72%) at 5 minutes after serotonin administration. The

Fig.1A.

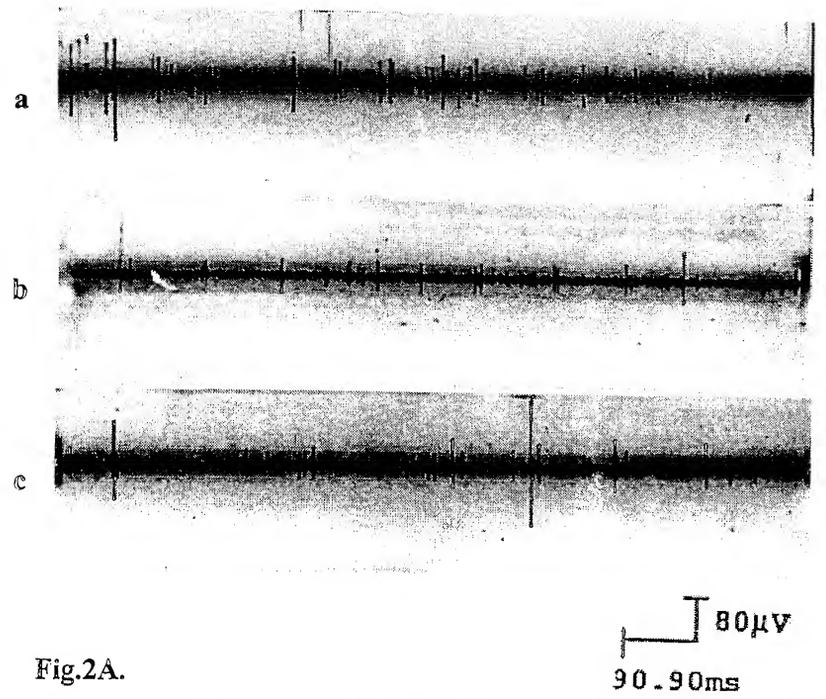

Fig.1B.

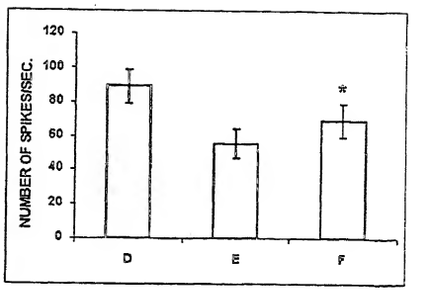

Fig.2A.

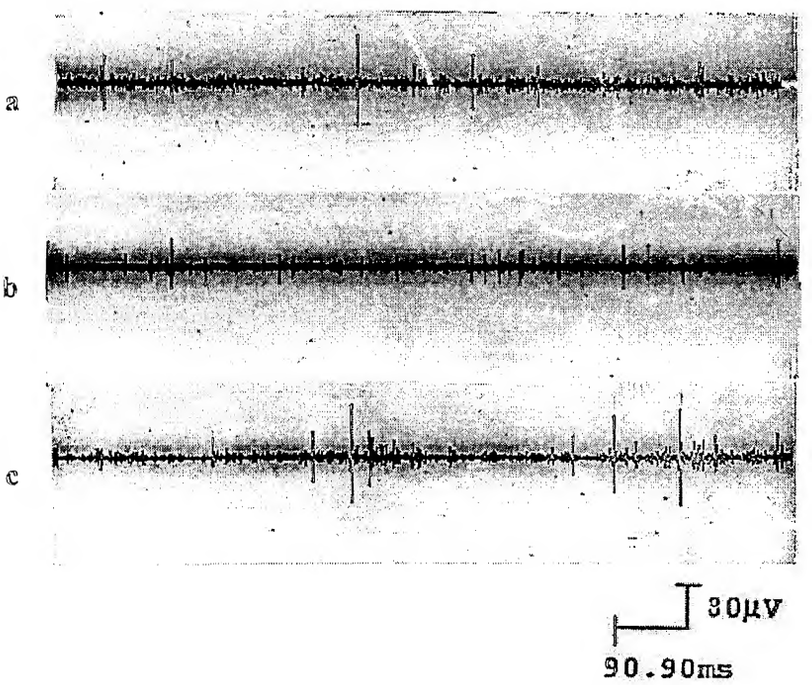

Fig.2B.

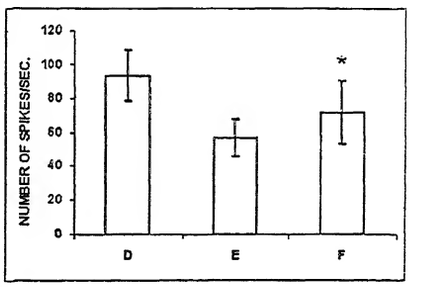

Fig. 1A– Effect of epinephrine (1 x 10-3M) on spontaneous electrical activity of the ventral nerve cord of scorpion, Heterometrus fulvipes. The recordings were made from the connectives between 1st and 2nd ganglia.

a. Control activity, b. Activity after perfusion with epinephrine for 10 minutes, c. Activity following ringer wash

Fig. 1B– Total activity as measured by number of spikes/min.

D. Control, E. Epinephrine, F. Ringer wash

Fig. 2A– Effect of norepinephrine (1 x 10^{-3}M) on spontaneous electrical activity of the ventral nerve cord of scorpion, *Heterometrus fulvipes*. The recordings were made from the connectives between 1st and 2nd ganglia.

a. Control activity, b. Activity after perfusion with epinephrine for 10 minutes, c. Activity following ringer wash

Fig. 2B– Total activity as measured by number of spikes/min.

D. Control, E. Epinephrine, F. Ringer wash

Fig.3A.

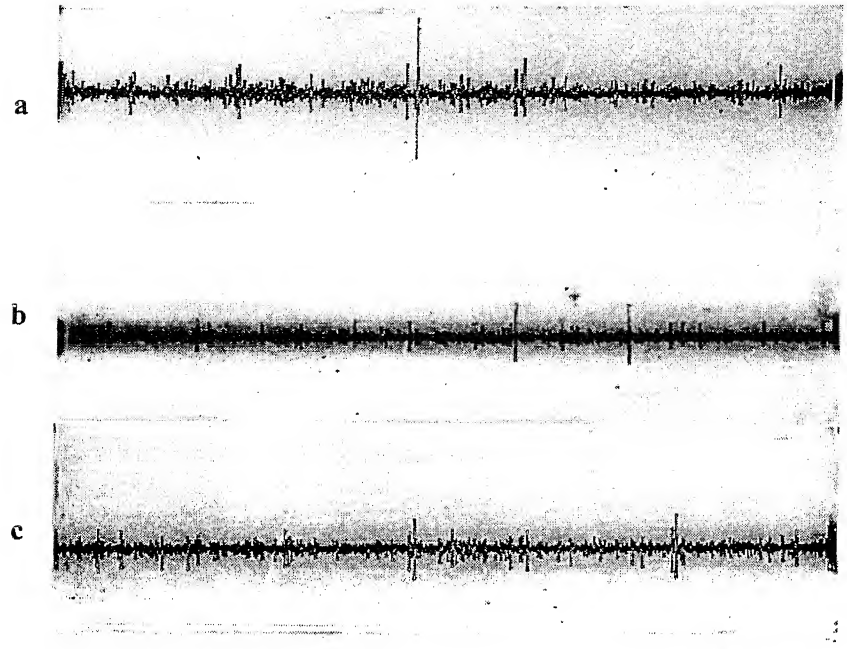

Fig.3B.

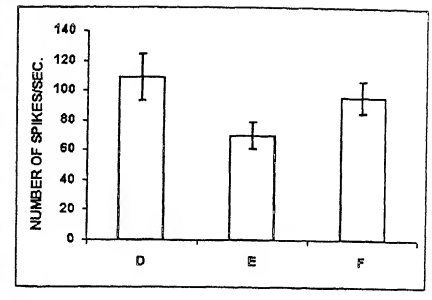

Fig.4A.

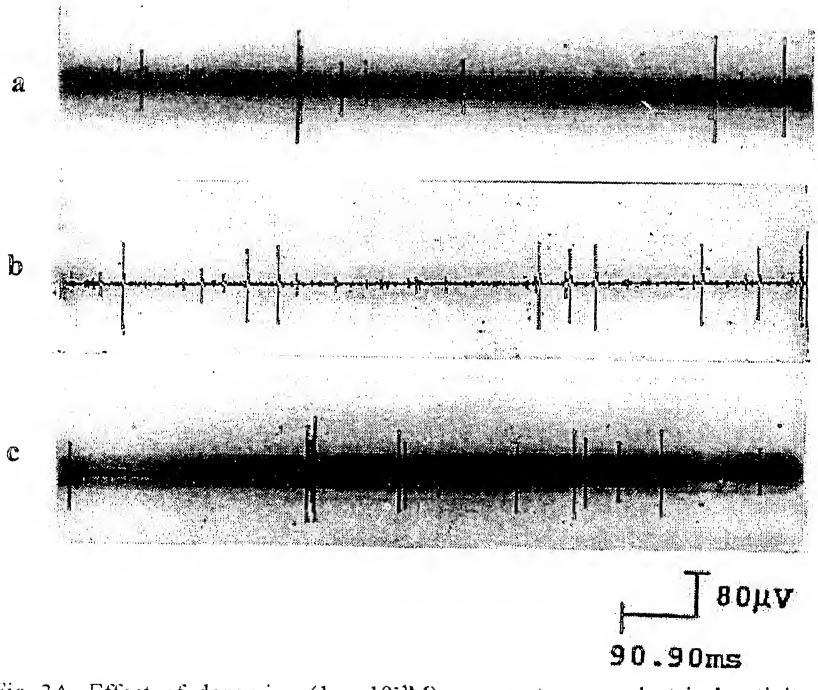

Fig.4B.

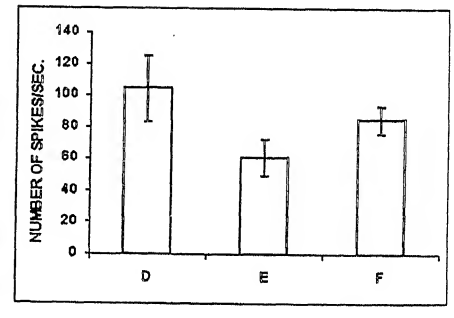

Fig. 3A– Effect of dopamine (1 x 10^{-3}M) on spontaneous electrical activity of the ventral nerve cord of scorpion, *Heterometrus fulvipes*. The recordings were made from the connectives between 1st and 2nd ganglia.

a. Control activity, b. Activity after perfusion with epinephrine for 10 minutes, c. Activity following ringer wash

Fig. 3B– Total activity as measured by number of spikes/min.

D. Control, E. Epinephrine, F. Ringer wash

Fig. 4A– Effect of serotonin (1 x 10^{-3}M) on spontaneous electrical activity of the ventral nerve cord of scorpion, *Heterometrus fulvipes*. The recordings were made from the connectives between 1st and 2nd ganglia.

a. Control activity, b. Activity after perfusion with epinephrine for 10 minutes, c. Activity following ringer wash

Fig. 4B– Total activity as measured by number of spikes/min.

D. Control, E. Epinephrine, F. Ringer wash

response of smaller fibers alone showed decrease while the large fibre responses did not show significant change. Recovery towards control levels was observed after ringer wash(Fig. 4A & 4B). The inhibitory action of the neurotransmitter and the recovery following ringer wash was found to be statistically significant.

The catecholamines dopamine, norepinephrine and epinephrine are neurotransmitters in a number of brain areas subserving functions relating to emotion, attention, and visceral regulation in vertebrates.[15] Scorpion nervous system has been shown to contain these catecholamines[11,13,12], although relatively little is known regarding their physiological role. Inhibitiory action of monoamines in the electrical activity of prosomian nervous system in the scorpion *Androctonus maritanicus* has been reported.[13] Norepinephrine has shown to have effects in crustacean central nervous system.[16] Such inhibitory action was also reported in the nervous system of other arthropods like stick insects[17] and *Limulus.*[18] Contrary to this, an increase in amplitude of giant interneuron-evoked excitatory postsynaptic potentials has been reported in the cockroach, *Periplaneta Americana.*[19] Sensitivity of olfactory receptor neurons of moths to biogenic amines has been reported.[10] Changes in levels of brain monoamines have been implicated in a variety of ontogenic shifts in behaviour in honeybee workers including onset of nest-guarding behaviour.[20] Action of monoaminergic systems in the central nervous system (CNS) has been implicated in the mediation of short-term and chronic physiological stress responses as well as aggressive and social dominance relationships in numerous taxa[21]. The presence of monoamines in the CNS of scorpions and the modulatory effect of monoamines on the electrical activity in the VNC in scorpion suggest that these neurotransmitters play an important role in the social and aggressive behaviour.

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Extension Lectures :

The Government of India has declared the year 2004 as 'Scientific Awareness Year' to cultivate the scientific temperament among the students and general mass so that a rational approach towards life is developed.

The Academy has been involved in 'Science Communication Programme' for the last several years and has now decided to spread the horizon of this programme through-out the country in the year 2004. Several activities have been planned for communicating science specially among the students and rural mass through the twelve Chapters of the Academy. In Allahabad, the Head Quarter has already started' Science Extension Lectures' under the experienced guidance of Prof. S.L. Srivastava, Co-ordinator, Science Communication Programme of the Academy. During August 2004 five (5) lectures have been held in different institutions of Allahabad.

On August 16, 2004 an informative and illustrative lecture was delivered by **Dr.(Mrs.) Sharda Sundaram** on **'Atoms, Molecules and Bonds'** in **Golden Jubilee Jagat Taran Inter College, Allahabad** for the students of +2 level.

On August 21, two lectures were delivered by the eminent scientists and fellows of the Academy. The first lecture was delivered by **Prof. (Miss) D. Kaul,** in **Gauri Pathshala Girls Intermediate College, Allahabad** on **'Basic and Advance Concepts in Genetics'** under which the structure of DNA, genes, recombinent DNA, replication, the mechanism of genetic dogma, gene therapy, cloning and use of genetics in the development of man kind were illustrated This field is also known as eugenics. The second lecture was in **Narayan Ashram Balika Inter College, Allahabad,** delivered by **Prof. S.L. Srivastava,** on **'Advances in Certain Novel Materials and Devices'** by which even the health services may be provided in each home.

On August 23, **Dr. Niraj Kumar,** Assistant Executive Secretary of the Academy delivered an interesting lecture in **M.L. Convent, Allahabad** on the **'Basic Concepts in Life Sciences.** He explained the origion of life, evolution of man and preventive and promotive role of nutrition.

On August 24, **Prof. U.C. Srivastava** of the **Zoology Department, A.U.,** delivered an informative lecture on **'Endocrine & Diseases'** in **Government Girls Inter College, Allahabad** for the students of class IX to XII. He explained the role of endocrine glands in regulation of metabolic pathways and many diseases.

All these lectures were very much appreciated by the students and teachers. Several lectures are planned during the next few months both in Allahabad and in nearby districts.

Professor Dwijesh Dutta Majumder, the pioneering and most renowned researcher in the field of cybernetics, computer science and information technology, former Head of ECSU and currently Professor Emeritus at Indian Statistical Institute, Kolkata, has been selected for Indian National Academy of Engineering **Life Time Contribution Award** in Engineering and Technology 2004.

24th Annual Convention of
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